Strategies for control of white blister disease in radish (*Raphanus sativus* L.) seed crops

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Abstract

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New Zealand produced radish seeds are exported to Continental Europe and Asia, with an export value exceeding $25 million in 2018. However, in recent years, white blister disease caused by the obligate biotrophic parasite *Albugo candida* has significantly reduced seed yields in radish crops. Growers currently use a fungicide seed treatment and multiple fungicide foliar sprays in an attempt to control the disease, but the effectiveness of this programme is variable.

Albugo candida is seedborne and oospores can be readily detected from the seed coat. However, whether the pathogen is present within the plant allowing vertical transmission via seed is not known. The presence of *A. candida* inside radish plant tissues was evaluated using PCR with one primer pair PKG-F and PKG-R. Pathogen DNA extraction was carried out using two methods: Chelex® resin and a Genomic DNA Mini Kit (Gene Aid, Taiwan). The latter was able to allow detection of *A. candida* DNA from all the types of radish tissue tested. *Albugo candida* was not detected inside radish plant tissues or seeds and contrary to previous reports, this strongly suggests the pathogen only exists on the plants externally, and on the seed coat. PCR was also used to identify *A. candida* on infected tissue of turnip and the common weed shepherd's purse. Whether the same race of *A. candida* occurred on all three plant species needs to be determined.

Fungicide seed treatments (Ridomil Gold MZ WG, Thiram and Iprodione) and non-chemical alternatives including hot water (50^{o}C for 15, 20, or 25 mins) and *Trichoderma atroviride* (strains LU132 and LU140) were evaluated for their ability to reduce the transmission of *A.*

candida from seed to seedling in two glasshouse experiments, one of which was overhead watered, and the other soil only watered. None of these treatments reduced disease transmission in either experiment. However, the disease incidence and severity were much higher in overhead watered plants, confirming that current irrigation methods for seed crops are contributing to disease spread and intensity within the crop.

A field trial in a radish seed crop was conducted in the 2018-2019 season, with eleven fungicide treatments (single products and combinations of products over the growing season). White blister disease severity was reduced by Ridomil Gold MZ WG, Cobra, Foschek plus Metalaxyl-M, Ranman plus Mancozeb plus Amistar followed by Ranman plus Mancozeb plus Seguris Flexi, then Ranman plus Mancozeb, then Ridomil Gold plus Pristine and finally Ridomil Gold, or Ranman plus Mancozeb followed by Ranman plus Mancozeb plus Seguris Flexi then Ranman plus Mancozeb plus Amistar, then Ridomil Gold MZ WG plus Pristine and finally Ridomil Gold MZ WG, but no treatments reduced the percentage of stagheads, infected racemes or infected pods per plant. Seed yield and quality were not increased by any of the treatments.

Seedborne inoculum is the most important source for establishment of white blister in New Zealand radish seed crops. Sourcing disease free seed, or finding on effective method for removing oospores from the seed coat before sowing, may be the best management method for reducing the impact of this pathogen in New Zealand. In the laboratory surface sterilising seeds using 5% sodium hypochlorite killed all oospores. Whether this can be used for an entire seed lot requires investigation.

Keywords: radish seed crops, *Raphanus sativus* L.*,* seedborne inoculum, seed quality, seed yield, seed treatment, *Albugo candida*, white blister, fungicide, *Trichoderma atroviride*, hot water, PCR, irrigation.

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Chapter 1

Introduction

1.1 *Albugo candida*

White blister is a disease caused by *Albugo candida* (Pel's. ex Hook.). *Albugo candida* is an obligate biotrophic parasite which belongs to the family Albuginaceae in the order Peronosporales of the class Oomycetes (Agrios, 2015). Although phylogenitically true fungi and oomycetes are different, *A. candida* causes a plant disease similar to rust fungi, and therefore the disease is known as white rust (white blister) (Holub *et al*., 1995; Kaur *et al*., 2011).

Albugo candida infects a large number of plant species including weed and vegetable crops, especially in the Capparaceae, Cleomaceae, Aizoaceae and Brassicaceae families (Saharan & Verma, 1992; Choi *et al*., 2009; McMullan *et al.,* 2015). Species include, but are not limited to, *Brassica juncea*, *B. oleracea*, *B. rapa, Raphanus sativus*, *Capparis spinose*, *Aubrieta deltoidea*, *Alyssum saxatile*, *Lunaria annua*, *Cleome hassleriana* and wild species such as *A. thaliana*, *Capsella bursa-pastoris*, *Sisymbrium officinale*, and *Cleome anomala* (Saharan & Verma, 1992; Jouet, 2016).

Minchinton *et al*. (2004) indicated that the first reports of the presence of *A. candida* on *C. bursa-pastoris*, *B. rapa*, *R. sativus* and *B. oleracea* were in 1894, 1895, 1903 and 1980, respectively in Australia. In 1995, 1996 and 1999, *A. candida* was first reported in *B. campestris* subsp. *nipposinica*, *B campestris* subsp. *narinosa, B. narinosa* and *Eruca sativa* in the U.S.A (Koike, 1996; Scheck & Koike, 1999). In Holland, *A. candida* was reported on cabbage and Brussels sprout (Gilijamse *et al*., 1998). The disease has reduced yield in *B. juncea* and *B. rapa* by up to 60% in India, Australia and Canada (Liu, 1992; Rimmer *et al*., 2000; Minchinton *et al*., 2004; Kaursu *et al*., 2008; Li *et al*., 2008; Awasthi *et al*., 2012; Bisht *et al*., 2016) and in other species in Africa, the U.K, Germany, Romania, Uruguay, New Zealand, Australia, the U.S.A, Canada, Mexico, Japan, China, Pakistan, Palestine, Turkey, Korea, India, etc. (CABI, 2017).

In New Zealand, *A. candida* was first recorded on *Lepidium sativum* in 1880 (Baker, 1955). Also, the presence of *A. candida* has been reported on *B. oleracea* (Baker, 1955; Hill, 1979; Pennycook, 1989), *B. campestris, B. rapa, C. bursa-pastoris, Cardamine* spp., *Cleome spinosa, Lepidium oleraceum, L. ruderale, Malcomia maritima, Rorippa islandica, Sisymbrium officinale*

(Baker, 1955) and *R. sativus* (Baker, 1955; Boesewink *et al*., 1977). White blister disease has spread to most radish seed production fields in New Zealand causing losses in seed yield of up to 70% (McKay, pers. comm., 2019).

1.1.1 Disease symptoms and cycle

1.1.1.1. Symptoms

White blister disease is recognised when sori (spore masses) appear with variable sizes and shapes on leaves, stems, inflorescences and pods of the plant (Fig. 1.1) (Saharan *et al*., 2014).

There are two types of infection, namely local and systemic (Sandhu *et al*., 2015). With local infection, the symptoms occur on leaves, stems and pods (Walker, 1957). The first visible symptom is the appearance of light yellow areas on the upper surface of the leaf (Fig. 1.2A). The lower-surface leaf shows the presence of white creamy sori one to three days after infection (Fig. 1.2B) (Sharma, 2016). Sometimes, the sori spread on both the upper and lower surface of the leaf. When the leaf is heavily infected, the entire leaf may become curled and distorted, and the pustules may appear over the entire surface (Fig. 1.2B). The sori are at first creamy in consistency and glossy white, but as the spores dry they become powdery in texture and turn to a grey colour. The leaf tissue on which the sori are borne remains alive for some days, but after this period a dead light brown area develops on the upper surface of the leaf above the clustered sori. When the infection is more general, the entire leaf blade withers (Kadow & Anderson, 1940).

The symptoms of systemic infection are infected inflorescences (staghead) (Fig. 1.1C) (Walker, 1957). The systemic infection often shows on young inflorescences (Asif *et al.,* 2017). The affected flowers are malformed, petals become green sepal-like and stamens may be transformed into a leaf-like or carpelloid structure. The petals and stamens persist in the flower instead of falling early. The stamens sometimes change into thick, club-shaped sterile bodies (Sharma, 2016). The ovules and pollen grains are usually atrophied, resulting in incomplete sterility (Kolte, 2018). The systemic infection causes greater yield loss than local infection (Pétrie, 1973; Verma & Petrie, 1980; Goyal *et al*., 1996; Sandhu *et al*., 2015).

In addition, *Albugo candida* may live within the host without causing any symptoms because it may develop an endophytic relationship with some cruciferous species (Jacobson *et al*., 1998; Patnude & Nelson, 2013).

Figure 1.1. Symptoms of infected radish seed crops of *Albugo candida* on lower leaves surface (A), stem (B), inflorescences (C) and pods (D). Photos by author.

Figure 1.2. Curled and distorted infected radish leaf in a radish seed crop: upper (A) and lower (B) leaves surface. Photos by author.

1.1.1.2. Life cycle

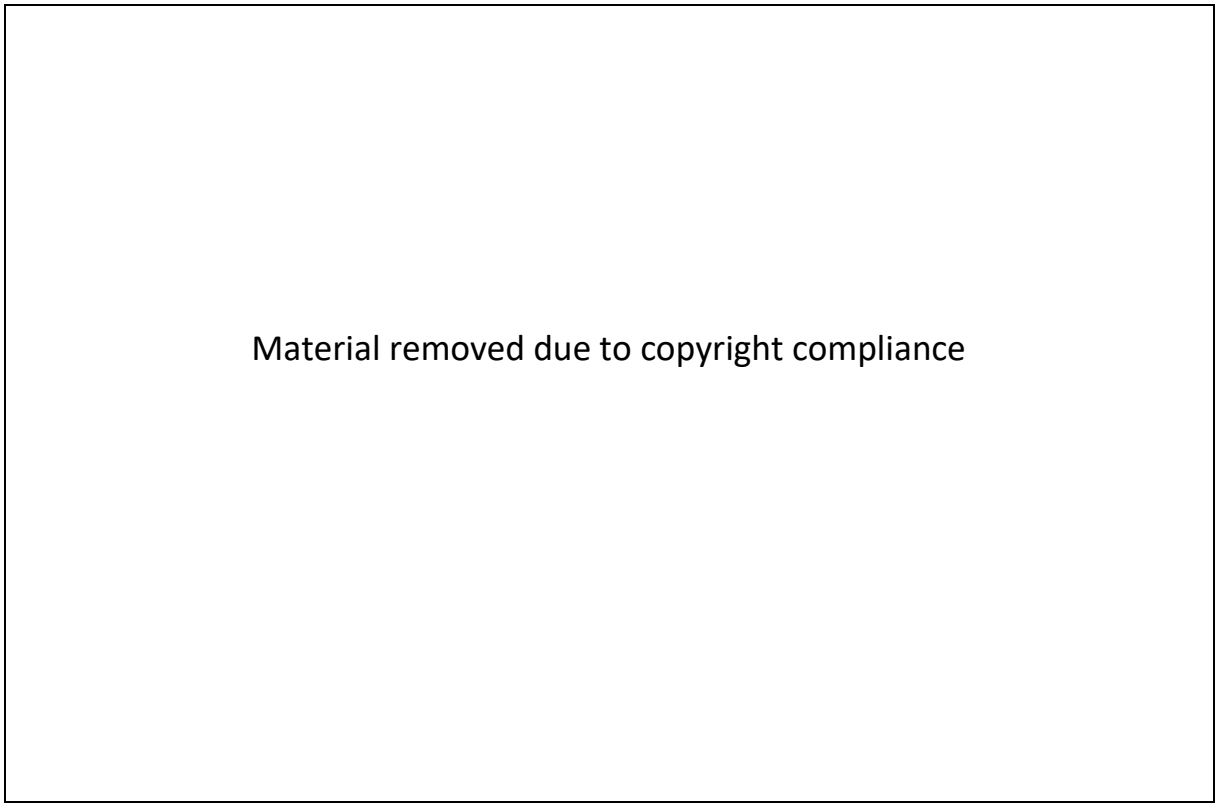


Figure 1.3. The life cycle of *Albugo candida* with asexual reproduction and sexual reproduction. A: hypha within host cell showing globular haustoria; B: infected leaf in vertical section showing sporangiophores and sporangial chains; C: germinating sporangium; D: sporangia releasing zoospores; E: zoospores; F: encysted zoospores; G: germination of encysted zoospores; H: antheridium and oogonium; I: plasmogamy; J: karyogamy; K: oospore; L: germination of oospore producing zoospores within vesicle; M: zoospores; N: germination of encysted zoospore. (Source: Alexopoulos *et al.,* 1996).

Albugo candida is a diploid organism that reproduces both asexually and sexually (Fig. 1.3) (Holub *et al*., 1995; Alexopoulos *et al.,* 1996; Agrios, 2015).

- ***Asexual reproduction***

Asexual reproduction takes place when zoospores are formed inside the sporangia. Firstly, the sporangiophores (Fig. 1.5A, B) produce chains of sporangia (Fig. 1.5C) under the host epidermis (Fig. 1.3B). The sporangium is smooth, double-walled and rounded (Fig. 1.3C). The formation of large numbers of sporangia in sporangiophores causes pressure which breaks the host epidermis, and hundreds of sporangia are seen on the host surface in the form of white creamy powder forming pustules (Fig. 1.1) (Saharan & Verma, 1992; Walker & Ven West, 2007). Sporangia then release zoospores (Fig. 1.3D). Zoospores then swim, encyst, germinate and infect new hosts (Fig. 1.3G) (Walker, 1957). Zoospore formation occurs within minutes and is considered one of the fastest developmental processes in any biological system (Walker & Van West, 2007; Heller & Thines, 2009; Meena *et al*., 2014; Cevik *et al*., 2019). The asexual cycle is repeated several times during the growing season (Pidskalny, 1984; Saharan & Verma, 1992).

- ***Sexual reproduction***

Sexual reproduction usually occurs in infected tissues. In the case of the inflorescence, this is often referred to as a "staghead" (Fig. 1.4). The oogonium is rounded and the club-shaped antheridium attaches to the side of the oogonium (Fig. 1.3H). When the outer periplasm of the oogonium wall becomes thick and dark, an oospore is formed (Fig. 1.6). The oospore is thick-walled and five-layered (Tewari & Skoropad, 1977). Oospores are yellow- brown, globose, 30 - 55µm in diameter, have a thick epispore and low, blunt irregularly branched ridges that may be smooth or curved (Wilson, 1907; Mukerji, 1975; Tewari & Skoropad, 1977). Oospores are carried on the seeds and can live over winter in the soil (Kadow & Anderson, 1940; Walker, 1957; Petrie, 1975; Verma *et al.,* 1975; Saharan & Verma, 1992). The oospores can germinate by producing germ tubes or by releasing up to 60 zoospores (Verma & Petrie, 1975; Pidskalny, 1984; McMullan *et al*., 2015). The zoospores are then liberated, swim and germinate to form germ tubes which then infect the host cell (Saharan & Verma, 1992).

Figure 1.4. Staghead infection of *A. candida* in a radish seed crop. Photos by author.

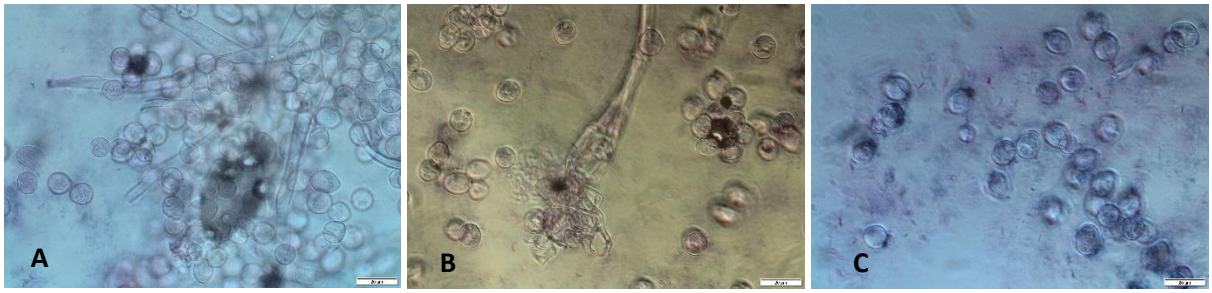


Figure 1.5. Sporangiophores of *A. candida* on an infected leaf (A), infected stem (B) and sporangia on staghead (C) in a radish seed crop, 200X magnification. Photos by author.

Figure 1.6. An oospore of *A. candida* on radish seeds, 1000X magnification. Photo by author.

1.1.2 Effects of abiotic and biotic factors on survival of *A. candida* and disease spread

Kumar & Kalha (2005) reported that white rust disease is sensitive to both abiotic and biotic factors.

1.1.2.1. Abiotic factors

Mycelium can survive during winter in infected crowns and lateral roots (Kadow & Anderson, 1940; Endo & Linn, 1960), becoming active and infecting new hosts in the spring.

The oospore has a thickly layered cell wall which allows it to live for a long time (Tewari & Skoropad, 1977). Agrios (2015) indicated that oospores are resistant to adverse environmental conditions and can survive in plant debris and soil for many years, or in infected seeds for a minimum of 20 years in dry storage conditions (Verma & Petrie, 1975; Tewari & Skoropad, 1977). However, Verma & Petrie (1975) and Verma *et al.* (1988) carried out experiments to check the presence of oospores which had been buried in infected soil for at least 6 months. They did not find any oospores. Thus, the soil may not be the primary source of *A. candida* inoculum (Saharan & Verma, 1992).

The overall temperature range for oospore germination is from 10^{o}C to 20^{o}C when relative humidity (RH) is above 70%, with the highest germination occurring at 13^{o}C on *B. campestris* inflorescences (Verma & Petrie, 1975; Minchinton *et al*., 2004; Mehta, 2014).

Sporangia have been reported to remain viable at -40^{o}C for 105 days in the dry powder form (Saharan & Verma, 1992). Infection was recorded at 30^{o}C, within 2 – 4 hours after attaching to the host. The ideal temperature for sporangia is 15^{o}C for four and a half days on *B. juncea* leaves (Lakra & Saharan, 1989).

Sporangial germination also happens through the production of zoospores which is more common than the production of germ tubes (Melhus, 1911; Napper, 1933; Lakra & Saharan, 1989). Sporangial germination requires temperatures from 10^{o}C to 20^{o}C for maximum germination (Napper, 1933; Endo & Lim, 1960; Lakra & Saharan, 1989; Walker & Van West, 2007). Lakra *et al.* (1989) observed that the exposure to light of 150 $\mu EM^{-2}s^{-1}$ postponed sporangial germination in *B. juncea* but Melhus (1911) and Holliday (1995) considered that sporangial germination was not impacted by light or darkness. Sporangia also need oxygen, a

film of water for germination, as well as a pH ranges of 5-7 (Uppal, 1926; Takeshita, 1954 as cited in Meena *et al.*, 2014; Lakra & Saharan, 1989; Lakra *et al.*, 1989).

Zoospores require temperatures between 1^{o}C and 18^{o}C with the ideal temperature for germination of 13^{o}C (Gilijamse *et al*., 1998). No zoospores are produced at 25^{o}C or higher (Mehta, 2014). Also, Walker & Van West (2007) reported water is necessary for the survival of zoospores.

Studies by Lakra & Saharan (1989), Harvey (2010), Minchinton (2012) and Arora *et al*. (2019) showed that the incidence of white rust was significantly increased under wet weather, wind, fog, rain splash or dews. In particular, infection increases significantly within three hours on wet leaves at 20°C (Gilijamse *et al*., 1998). Young plants are more readily infected than mature plants (Kumar *et al*., 1995; Harvey, 2010). Verma & Bhowmilk (1988) and Mehta (2014) determined that the ideal conditions for *A. candida* development in *B. juncea* was below a 16^{o}C mean temperature, wind speed from 2-4 km/h and above 70% RH. Staghead formation requires a temperature between 6^{o}C and 25^{o}C, RH above 60% and a mean rainfall of 160mm in Indian mustard (Saharan & Verma, 1992; Kaur *et al.*, 2006).

Petrie (1975) suggested that the disease spread could be by oospores on contaminated seeds and Jacobson *et al*. (1998) also stated that the disease is transmitted by infected seeds.

1.1.2.2. Biotic factors

Petrie & Vanterpool (1974) mentioned some 20 other fungi, including several crucifer pathogens, found to be associated with staghead and stem and pod infections caused by *A. candida* on *B. campestris*, *B. kaber* and *Camelina microcarpa*. For example, an association or mixed infection between *A*. *candida* and *Hyaloperonospora parasitica* pathogens on different parts of the crop such as the stems, leaves or inflorescences is known (Bains & Jhooty, 1979; Saharan & Verma, 1992). On the other hand, spores of *A*. *candida* and *H*. *parasitica* do not spread at the same time (Bains & Jhooty, 2008). An experiment on Indian mustard showed that if the India mustard was inoculated previously with an incompatible pathogen, it was resistant to *H*. *parasitica.* But if it had been inoculated previously with compatible *A. candia,* it was sensitive to *H*. *parasitica* (Singh *et al*., 2002). The favourable conditions for mixed infection of *A*. *candida* and *H. parasitica* were temperatures of 14-15^{o}C, RH above 65% and mean rainfall above 152mm (Bains & Jhooty, 1979).

1.1.3 Races of *Albugo candida*

Table 1.1. Reported races of *A. candida* in the world (Minchinton *et al*., 2004; Saharan 2010; Minchinton, 2012; Meena *et al*., 2014).

Race name	Source	Primary host's specific name	Reference
1	North America	*Raphanus sativus*	Pound & Williams, 1963
2	North America	*Brassica juncea*	Pound & Williams, 1963
2A	Western Australia	*B. juncea* cv. Vulcan; Commercial Brown	Kaur *et al*., 2008
2V	North America Western Australia	*B. napus* *Raphanus raphanistrum*	Petrie, 1994 Kaur *et al*., 2008
3	North America	*Armoracia rusticana*	Pound & Williams, 1963
4, AcEm2	North America	*Capsella bursa-pastoris*	Pound & Williams, 1963; Borhan, 2008
5	North America	*Sisymbrium officinale*	Pound & Williams, 1963
6	North America	*Rorippa islandica*	Pound & Williams, 1963
7	North America	*B. rapa; B. campestris*	Verma *et al*., 1975
7A	North America	*B. rapa* var. Oleifera	Pidskalny & Rimmer, 1985
7V	North America	*B. rapa* cv. Reward	Petrie, 1994
8	North America	*B. nigra*	Delwiche & Williams, 1977
9, AcBoT, AcBoL	North America	*B. oleracea*	Williams, 1985; Hill *et al*., 1988; McMullan, 2015; Jouet, 2016
10	North America	*Sinapis alba, B. kaber*	Williams, 1985; Hill *et al*., 1988
11	North America	*B. carinata*	Williams, 1985
12	India	*B. juncea*	Verma *et al*., 1999
13	India	*B. rapa* var. Toria	Verma *et al*., 1999
14	India	*B. juncea* cv. RL 1359	Gupta & Saharan, 2002
15	India	*B. juncea* cv. Kranti	Gupta & Saharan, 2002
16	India	*B. juncea* cv. Kranti	Gupta & Saharan, 2002
17	India	*B. juncea* cv. RH 30	Gupta & Saharan, 2002
18 to 34	India	*B. juncea* cv. RH 30*;* EC 182925; DVS 7-3-1	Jat, 1999
35 and 36	India	*B. rapa* var. Brown Sarson	Jat, 1999
37	India	*B. nigra*	Jat, 1999
1 to 9	India	*Brassica* species	Singh & Bhardwaj, 1984
1 to 5	India	*Brassica* species	Lakra & Saharan, 1988

Many different races of *A. candida* have been reported on different *Brassica* species from different parts of the world (Table 1.1).

Pounds & William (1963) indentified races 1, 2, 3, 4, 5 and 6 of *A. candida* from *R. sativus, B. juncea, A. rusticana, C. bursa-pastoris, S. officinale* and *R. islandica*, respectively. Singh & Bhardwaj (1984) reported nine races from *B. campestris* var. Brown Sarson, *B. campestris* var. Toria, *B. juncea* and *B. campestris* var. Pekinensis, while Lakra & Saharan (1988) found five races from *R. sativus* (race 1)*, B. juncea* (races 2 and 3)*, B. chinensis* (race 4) *and B. tournifortii* (race 5). Borhan (2008) found race 4 (AcEm2) from *C. bursa-pastoris*. Kaur *et al*. (2008) identified races 2A and 2V in Western Australia from *B. juncea* cv. Vulcan, *B. juncea* cv. Commercial Brown and *R. raphanistrum,* respectively. Pidskalny & Rimmer (1985) and Petrie (1994) recognized races 7A and 7V from *B. rapa* var. Oleifera and *B. rapa* cv. Reward, respectively. Williams (1985) indentified races 9, 10 and 11 from *B. oleracea, S. alba* and *B. carinata.* Similarly, Hill *et al*. (1988), McMullan (2015) and Jouet (2016) found races 9, AcBoT and AcBoL from *B. oleracea.* Hill *et al*. (1988) also found race 10 from *B. kaber.* Verma *et al*. (1999) indentified two races, 12 and 13, from *B. juncea* and *B. rapa* var. Toria, respectively in India. Gupta & Saharan (2002) reported four races of *A. candida* from different species of *B. juncea.* Once again, *B. juncea* spp. are also identified as the hosts of races from 18 to 34 (Jat, 1999). Jat (1999) reported races 35 and 36 belong to *B. rapa* var. Brown Sarson.

To distinguish the races of *A. candida*, Pounds & William (1963) divided races according to the diameter of sporangia. For example, races 1, 2, 3, 4, 5 and 6 were 16.8, 17.8, 17.5, 15.2, 19.9 and 17.5µm, respectively. However Lakra & Saharan (1988) identified race 2 and race 3 by observing the size of the pustules on *B. juncea*. In the same way, Gupta & Saharan (2002) also reported four races on *B. juncea* were recognised by the infection symptoms on the plant (Table 1.2). They described four races which had similar symptoms on the lower leaf surface but they differed for pinhead size, shape of the pustule, pustule size and symptoms on the upper leaf surface (Table 1.2).

To date, only race 1 has been reported from *R. sativus* (Pounds & William, 1963), while race 2V has been reported from *R. raphanistrum* (Kaur *et al*., 2008). Whether the *A. candida* found on radish in New Zealand is also race 1 has yet to be established.

Table 1.2. Distinguishing *A. candida* races by symptoms (Gupta & Saharan, 2002).

Race name	Pinhead size	Pustule	Pustule size (mm)	Lower Leaf surface	Upper leaf surface
1	Small	Creamy, raised	1-2	Margin	Pinhead type green island without halo zone
2	Big	Bright, raised	1-2	Margin	No green island
3	Medium	Creamy, raised with one concentric ring having pin head type dot in centre	3-4	Margin	Light and green island without halo zone
4	Medium	Circular, creamy white, raised with two concentric rings	5	Margin	Violet island without halo zone

1.1.4 Effects of *Albugo candida* on *Brassica* crops

White blister has caused yield losses from 20 to 90% in *Brassica* crops throughout the world (Asif *et al.,* 2017). In Pakistan, *A. candida* became a major problem, causing 60-90% yield losses in *B. napus* (Asif *et al*., 2017). In the same crop, 60% and 10% yield losses were found in Canada (Bernier, 1972; Petrie & Vanterpool, 1974) and Western Australia (Barbetti, 1981), respectively. Petrie (1973) estimated economic losses of 1.7, 4.1 and 2.4 million dollars for 1970, 1971 and 1972, respectively in *B. campestris* in Canada caused by *A. candida*.

In mustard, *A. candida* caused a yield loss of 17 - 55% (Saharan *et al*., 1984; Kumar, 2009). Furthermore, when *A. candida* was associated with *H. parasitica,* the yield losses were from 17% to 90% in *B. juncea* because of staghead infection (Bains & Jhooty, 1979; Lakra & Saharan, 1989).

Disease incidence of white blister disease in Brussels sprouts reached 80% in the U.K (Appleton, 1979). Etebarian (1993) reported *A. candida* infection of 8 -27% in radish and 30-47% in cress in Iran.

1.2 Disease management

A number of studies have been undertaken to evaluate methods for control of *A. candida*. These are summarised in the following sections.

1.2.1 Cultural control

Savulescu (1957) and Saharan & Verma (1992) showed that removing and burning infected plant tissue, weeds and debris from the field could stop the formation of oospores. Also modifying the environment is another method to control *A. candida*. Because sporangial germination requires free water to germinate (Uppal, 1926; Lakra & Saharan, 1989; Lakra *et al.*, 1989) and not just high humidity, minimizing the occurrence and duration of leaf wetness, for example by using the drip-irrigation system, can reduce infection (Mehta, 2014). Minchinton *et al*. (2013) indicated overhead irrigation in the morning from 8AM to 12PM showed lower disease incidence on leaves in radish than in the evening (8PM – 12AM) in Australia because the zoospores released during the day could survive until irrigation in the evening, allowing infection of the wet leaves (Gilijamse *et al*., 2004). *Albugo candida* is dispersed readily by wind (Saharan & Verma, 1992) and therefore planting windbreaks around or within fields can reduce the ability of the fungus to spread aerially among hosts (Patnude & Nelson, 2013).

Soil should be ploughed to a depth of 15-25 cm to postpone the oospores infection from the soil and debris under frequent irrigation. Mehta (2014) found oospores at a depth of 15 cm infected the host plants later than at the depth of 7.5 cm.

Khangura & Sokhi (1994) reported a lower *A. candida* infection in Indian mustard well supplied with the nutrients phosphorus, magnesium, nitrogen, sulphur, calcium, iron and manganese, than in a crop with high potassium, copper and zinc, similar to a previous report by Saharan & Verma (1992). Thus, using proper fertilizer should be considered for control of *A. candida*.

Timely planting of crops was found to be important for the reduction of staghead formation in Western Australian crops of rapeseed (Barbetti, 1981; Minchinton *et al*., 2004). For example, the disease severity and staghead infection of mustard were lower in an October sowing than in a November sowing in India (Dange *et al.*, 2003; Kaur *et al*., 2006; Kumar, 2009; Mehta, 2014; Magar *et al*., 2017). The later the sowing, the higher disease severity and incidence (Magar *et al*., 2017). Planting crops in drier seasons can reduce infections and the dispersal of the pathogen as the disease is more prevalent in areas of high humidity or in moderately wet climates (Patnude & Nelson, 2013).

There are more than 200 host plant species of *A. candida* (Jacobson *et al*., 1998). Rotation of crucifers with non-hosts of the pathogen for at least three years is highly recommended

(Patnude & Nelson, 2013; Mehta, 2014). Also, seed health should be assessed before sowing to ensure no oospores are on the seeds (Mehta, 2014).

1.2.2 Resistance sources

Sources of resistance have been found for several pathotypes, but only a few studies reported for *A. candida*. Minchinton *et al.* (2013) reported resistant varieties could reduce disease incidence by up to 99%, increase yield by 10% and increase profit by 12%.

There is a single dominant gene for *A. candida* resistance (Tiwari *et al.*, 1988; Singh & Singh, 1988 as cited in Sahara & Verma, 1992; Franke *et al.,* 1999; Gugel *et al.,* 1999; Vignesh *et al*., 2009). Cultivars of *B. juncea* were used as known resistance sources to *A. candida* race 2, but *A. candida* race 2V which is more virulent was also detected in *B. juncea* spp. (Franke *et al.,* 1999). *Brassica napus* is resistant to both races 2A and 2V (Gugel *et al.,* 1999). Thus, the successful backcrossing of *B. juncea* and *B. napus* provided resistance to *A. candida* race 2V in *B.juncea* (Franke *et al.,* 1999; Gugel *et al.,* 1999). Moreover, Singh & Singh (1988) as cited in Sahara & Verma (1992) and Vignesh *et al*. (2009) found that the hybrid interspecific crosses of *B. carinata* and *B. juncea* could prevent *A. candida*, similar to a report by Tiwari *et al.* (1988). Those authors demonstrated that inheritance of resistance to *A. candida* race 2 in *B. juncea* was easily transferred and adapted to genotype through backcrossing.

In *B. napus*, there were eighteen resistant cultivars (Bharadwaj & Sud, 1993 as cited in Sharma, 2016), three resistant cultivars in India (Yadav & Sharma, 2004) and resistant genotypes Tower, GS-7027, HNS-4, HNS- 10, Midas, Norin (Dang *et al*., 2000), GSL-1 (Pandey *et al.,* 2014), DLDC-1, DRMR-100, DRMR-312, EC-339000, GSL-1, NPJ-158, RH-0644 (Bisht *et al*., 2016) for *A. candida*.

In *B. carinata,* thirteen cultivars (Bharadwaj & Sud, 1993 as cited in Sharma, 2016), two cultivars (Yadav & Sharma, 2004), genotypes PBC 9921 (Kumar & Kalha, 2005), NPC-12 (Vignesh *et al*., 2009) and PBC-9221 (Pandey *et al.,* 2014) showed high levels of resistance to *A. candida.*

Brassica campestris cv. Tobin is a resistant variety to *A. candida* race 7 in Canada and India (Saharan & Verma, 1992). Moreover, genotypes BSH- 1, Chamba, Gulivar, Sangam, SSK-1, TH-68 (Dang *et al*., 2000) and two cultivars of *B. campesris* were also resistance sources (Yadav & Sharma, 2004).

In *B. juncea,* over 40 genotypes resistant to *A. candida* have been reported (Raj *et al*., 1997; Dang *et al*., 2000; Gupta *et al*., 2002; Vignesh *et al*., 2009; Pandey *et al.,* 2014). Also, 28 resistant cultivars of *B. juncea* were reported by Yadav & Sharma (2004) and Barbetti *et al.,* (2016). In particular, genotypes PT 303, PCRS and CCYS7B had resistances against both white rust and *Alternaria* blight diseases (Raj *et al*., 1997).

Awasthi *et al.* (2012) reported all lines of *B. juncea* var. *cutlass* were resistant to *A. candida.* Another eight genotypes of *B. juncea* were found to be resistant to *A. candida* race 2V (Awasthi *et al.,* 2012). Li *et al.* (2008) reported genotypes CBJ-001, CBJ-003 and CBJ-004 of *B. juncea* from China consistently showed high levels of resistance to *A. candida* race 2 on leaves.

Petkowski *et al.* (2010) found *B. oleracea* var. *capitata* was immune to the *A. candida* race 7 and 9 in Australia. Also, *B. oleracea* var. Tyson reduced disease incidence by up to 99% in Australia (Minchinton *et al.*, 2013).

Williams & Pound (1936), Humaydan & Williams (1976) as cited in Saharan & Verma (1992) and Bonnet (1981) confirmed that the white rust resistance to *A. candida* race 1 in *R. sativa* spp. was Ac1 – a single dominant gene. Delwiche & Williams (1981) demonstrated that the single dominant gene Ac2 resists *A. candida* race 2 in *B. nigra.* Fan *et al*. (1983) found that the inheritance of *A. candida* resistance in *B. napus* cv. Regent was conditioned by independent dominant genes at three loci namely Ac7-1, Ac7-2 and Ac7-3. The resistance was conferred by dominance at any one of the three loci and plants with recessive alleles at all loci were susceptible.

Borhan *et al*. (2008) reported that *Arabidopsis thaliana* contained *white rust resistance 4 (WRR4)* a gene on chromosome 1 encoding a cytoplasmic toll-interleukin receptor-like nucleotide- binding leucine-rich repeat receptor-like (TIR – NB - LRR) protein which can confer resistance to races 2, 4, 7 and 9 of *A. candida* in *B. juncea*, *C. bursa-pastoris, B. rapa* and *B. oleracea,* respectively. *Arabidopsis thaliana* accessions provided wide species resistance to *A. candida* race 2 in *B. juncea*, 2V in *B. juncea*, 9 and AcBoT in *B. oleracea* (Cevik *et al*., 2019). Arora *et al*. (2019) succesfully found gene *BjuWRR1,* a single coiled-coil (CC) – NB - LRR protein encoding R gene of *B. juncea*, is a resistant gene to *A. candida*.

1.2.3 Fungicide control

Copper fungicides were orgininally used to control *A. candida*. They were useful to control leaf infection, but unsuccessful for control of stagheads (Saharan & Verma, 1992). Stone *et al*. (1987) and Lakra & Saharan (1988) found metalaxyl fungicides helpful to control the pathogen on *B*. *campestris,* providing up to 95% reduction of leaf infection. Other fungicides which have been used to control the disease include chlorothalonil, mancozeb, copper oxychloride, zineb, thiophanate-methyl, azoxystrobin, cyazofamid, benomyl, triphenyl stannanol, tridemorph, pristine, trifloxystrobin and kresosim methyl (Verma & Petrie, 1979; Srivastava & Verma, 1989; Minchinton *et al*., 2004; Mehta, 2014; FAR, 2017; Braithwaite *et al*., 2018; Gairola & Tewari, 2019). Mehta (2014) recommended the best control of stagheads was by three sprays of metalaxyl followed by mancozeb with at least two sprays at flowering time.

Mixed fungicides often centred on metalaxyl have also been effective against *A. candida* (Mathur & Bhatnagar, 1991; Minchinton *et al*., 2004; Braithwaite *et al*., 2018). For example, the combination of metalaxyl-m plus mancozeb or boscalid plus pyraclostrobin significantly increased radish seed yield in an infected crop (Braithwaite *et al*., 2018). Also, the combination of azoxystrobin and metalaxyl plus mancozeb was found to be the most efficient foliar spray for control of *A*. *candida* on horseradish and Indian mustard crops (Minchinton *et al*., 2004; Gairola & Tewari, 2019). The combination of propiconazole and tebuconazole + trifloxystrobin was also recommended (Gairola & Tewari, 2019). FAR (2017) showed an application of Seguris flexi plus Azoxystrobin or a rotation of Azoxystrobin followed by Ridomil plus Azoxystrobin reduced raceme infection and increased seed yield significantly. A rotation of metalaxyl plus mancozeb followed by Antrocol was suggested for control of *A. candida* (Asif *et al*., 2017).

Control of white rust of radish was most effective when four sprays of Difolatan 0.3%, Daconil 0.1%, Dithane M-45 0.2%, Ridomil 0.1% or Aliette 0.1% were applied at 8-10 day intervals (Glaeser, 1973; Holtzhausen, 1978; Sharma & Soni, 1982; Saharan & Verma, 1992). Bhatia & Gangopadhyay (1996) showed the foliar fungicide Ridomil (0.144% a.i) sprayed at 50, 65 and 80 days after sowing (DAS) in rapeseed and mustard reduced disease severity and had the highest seed yield.

Kumar (2009) reported foliar spray application of benzothiadiazole 0.02% (Bion 50WG®) at 30 and 60 DAS lowered leaf and staghead infection to 17.5% and 1.9%, respectively, because benzothiadiazole activated the defense response of plants against *A. candida*. Similarly, Kaur

& Kolte (2001) demonstrated that benzothiadiazole reduced white blister disease by up to 61%.

Fungicide products registered by the U.S. Environmental Protection Agency and the Hawai'i Department of Agriculture for control of *A. candida* are listed in Table 1.3 (Patnude & Nelson, 2013). Depending on the type of infection, a specific fungicide and application method should be selected.

Table 1.3. Some fungicides registered for use against *A. candida* in Hawai'I (Patnude & Nelson, 2013).

Product Name	Active Ingredient	Formulation	EPA Registration Number	Suggested Crop Use
Ridomil Gold® SL	Metalaxyl-M	Emulsifiable concentrate	100-1202	Broccoli, cabbage, cauliflower, Chinese broccoli, Chinese cabbage, radish
Serenade® MAX	*Bacillus subtilis* strain QST 713	Wettable powder	69592-11	Broccoli, cabbage, cauliflower, watercress
Abound® Flowable Fungicide	Azoxystrobin	Flowable concentrate	100-1098	Daikon, watercress
Champion® WG Agricultural Fungicide	Copper hydroxide	Wettable powder	55146-1	Broccoli, cabbage, cauliflower, watercress
Earth-tone® Garden Fungicide	Octanoic acid, copper salt	Solution - ready to use	67702-1-83598	Broccoli, cabbage, cauliflower
Regalia® Biofungicide Concentrate (organic)	*Reynoutria sachalinensis*	Emulsifiable concentrate	84059-3	Broccoli, cabbage, cauliflower, bok choy, watercress

1.2.4 Fungicide seed treatment

Seed treatment is a method to apply chemicals (fungicides and insecticides), biological products, heat or electricity to seeds to prevent seed-borne disease. Seed treatment with Apron 35 SD protected *B. campestris* from white rust infection for at least 60 days in Canada. It killed zoospores on emergence or prevented oospore germination (Verma & Petrie, 1979; Saharan *et al*., 2014). Stone *et al*. (1987) recommended that seed dressing or soil drenching were good ways to reduce primary infections from germinating oospores. Seed dressing alone with metalaxyl at 5.0 g a.i./kg, or with soil drench applications, reduced oospores and sporangial inoculum on the foliage during the growing season when combined with a foliage

spray application of metalaxyl of 2.0 kg a.i. /ha or higher. Foliar applications also reduced staghead infections. Seed dressings only provide protection for a limited period of time, and if conditions favour disease development throughout the season, staghead development will not be controlled. By providing early disease control, however, seed treatment could reduce the secondary inoculum potential in the crop, and thereby limit the initiation of stagheads from newly infected flower buds (Meena *et al*., 2014).

Bhargava *et al*. (1997) found that the most efficient method for white blister disease control in mustard was to treat seeds with metalaxyl and then use subsequent combinations of sprays of chlorothalonil and mancozeb. Similarly, a combination of seed treatment with metalaxyl and three foliar sprays of mancozeb was effective (Minchinton *et al*., 2004). Bhargava *et al*. (1997) treated seeds with metalaxyl SD-35 then sprayed with chlorothalonil 75 WP (0.1 %) at 20, 40, and 60 DAS, or with Ridomil MZ-72 at 20, 40 and 60 DAS. Moreover, metalaxyl (Apron 35) seed treatment followed by a foliar spray of Ridomil at 50 DAS controlled white blister disease in mustard more effectively than at 65 DAS (Bhatia & Gangopadhyay, 1996). Mehta (2014) suggested seed treatment with metalaxyl (Apron SD-35) and three sprays of mancozeb (Dithane M-45) starting at 60 DAS because metalaxyl can prevent mustard crops from white blister infection for at least 60 days (Mehta, 2014).

1.2.5 Hot water seed treatment

Hot water seed treatment is another method to control seedborne pathogens of Brassica crops (McLean, 1947; Williams, 1967; Holtzhausen, 1978; Nega *et al*., 2003; Toporek & Hudelson, 2017). These authors suggested that seed treated with hot water at 50^{o}C for from 15 to 25 minutes controlled seedborne *Alternaria* species effectively. However, there is a lack of research on hot water seed treatment for *A. candida*.

1.2.6 Biological control agents (BCAs)

Fungicides are an important part of disease management in developed countries. However, there is concern over both the environmental impact of these toxic compounds and their effect on human health (Hernández *et al*., 2013). More study is required to ascertain the long term effects of many fungicides. The registration of products has occurred in the past where research has found a significant risk to human health or deleterious environmental impacts (O'Brien, 2017). Consumers have also put pressure on the agricultural sector to provide food products that have had less chemical exposure. This has led to a significant amount of research

into using biological alternatives for disease control. The main advantages of using a biological control (biocontrol) agent are that they are generally considered better for the environment and often cause less collateral damage to other organisms (O'Brien, 2017).

Trichoderma spp., *Bacillus subtilis*, *Coniothyrium minitans, Aspergillius versicolor,* etc. are known as good BCAs against plant pathogens (Patnude & Nelson, 2013; O'Brien, 2017) and some of the effective biocontrol strains of these genera can be used as biological fungicides. In Hawai'i, one product registered for control of *A. candida* is a biological fungicide (Table 1.3) (Patnude & Nelson, 2013).

Trichoderma harzianum used as a seed treatment at 10 g/kg seed also reduced white bister disease severity on Indian mustard leaves (Meena *et al*., 2011). However, a combination of *T. harzianum* (10 g/kg seed) seed treatment and foliar spraying with *Pseudomonas fluorescens* (10 g/l water), and a combination of *T. harzianum* (10 g/kg seed) as a seed treatment and foliar spraying with *T. harzianum* (10 g/l water) provided better control than the seed treatment alone (Meena *et al*., 2011).

1.2.7 Biofungicides

Kumar (2009) applied 1% extracts of *Eucalyptus,* neem (*Azadirachta indica*) and garlic (*Allium sativum*) at 60, 75 and 90 DAS when the first disease symptoms appeared. *Eucalyptus* was the most effective in reducing leaf and staghead infection of *A. candida* in mustard crops to 18.3% and 3.7%, respectively, followed by *A. indica* (19.9% and 4.0%), and *A. sativum* (21.0% and 3.9%). *Allium sativum* extract significantly reduced white blister disease severity in mustard as well as increasing seed yield (Meena *et al*., 2011; Gairola & Tewari, 2019). Meena & Jain (2002) showed that extracts of *A. indica, Ocimum sanctum* and *Datura stramonium* reduced *A. candida* infection. Also, *Reynoutria sachalinensis* extraction is registered for control of *A. candida* in Hawai'I (Table 1.3) (Patnude & Nelson, 2013).

1.2.8 Disease prediction tool

Brassica$_{spot}$™ is a system using software MORPH (Methods of Research Practice in Horticulture) to predict foliar diseases in Brassica crops in some countries in Europe. The Brassica$_{spot}$™ system comes from the UK (Kennedy & Gilles, 2003 as cited in Minchinton, 2007). The Brassica$_{spot}$™ system will use data from a weather station placed in or near the crop field. According to this forecasting data, the growers will make a decision with their

fungicide and irrigation applications. In the U.K, the Brassica$_{spot}^{TM}$ system helped the growers to reduce fungicide applications for control of foliar diseases by up to 50% in Brussels sprouts (Kennedy & Gilles, 2003 as cited in Minchinton, 2007).

In Australia, the Brassica$_{spot}^{TM}$ system has been used to predict white blister disease in Brassica crops such as broccoli, Chinese cabbage, Brussels sprouts and radish. Use of the Brassica$_{spot}^{TM}$ system to help make the decision for disease control decreased disease incidence between 72 and 83% as well as increasing farm profit by up to 12% per hectare (Minchinton *et al.,* 2013).

1.3 Radish crops

Radish (*Raphanus sativus* L.) (2n = 2x = 18) is a root vegetable crop in the Brassicaceae family. Radish was known in China in 500 BC, the Mediterranean before 2000 BC and Japan about 1000 years ago (CABI, 2018; CGIAR, n.d.). According to Pistrick (1987), the cultivated species may have been grown in the eastern Mediterranean and the adjacent Near East for vegetable (leaf, root) or oil use (seed) more than 4000 years ago. Nowadays, radish is planted throughout the world (CABI, 2018).

Compared to other vegetable species, radish also has a wide range of varieties, sizes, colours, shapes and cultivation time to meet the demands of consumers or processors. Radish varieties were divided into three groups by Pistrick (1987):

- ✓ convar. *oleifera* (*R. sativus* var. *oleiformis*) known as *R. sativus* Leaf radish group: oilseed and fodder radishes, grown in Southeast Asia and in Europe for leaf fodder and as green manure.

- ✓ convar. *caudatus* (*R. sativus* var. *caudatus*) known as *R. sativus* Rat-tailed radish group: grown for its edible immature green or purple seed pods and leaves. This type is grown primarily in Southeast Asia.

- ✓ convar. *sativus* (*R. sativus* var. *sativus*) known as *R. sativus* Small radish group: all forms have edible roots, leaves and germinated radish sprouts with many different varieties but generally of the small type.

- ✓ Moreover, *R. sativus* var. *niger is* known as *R. sativus* Chinese radish group with the common names Chinese radish, Japanese radish and Oriental radish as the fourth group (CGIAR, n.d.).

Radish production in the world is approximately 7 million tonnes per year, accounting for 2% of total vegetable production (Schippers, 2004). In India, radish seed yields ranged from 0.9 to 1.8 t/ha (Malhotra & Chaudhry, 2001; Warade *et al.*, 2004; Shukla *et al.,* 2012). Radish is an important crop and food in China, Japan, Yemen, India, Nepal, Bhutan, and Korea (Yamaguchi & Okamoto, 1997; Schippers, 2004; Gin & Lee, 2006; Zaki *et al*., 2012; CABI, 2018). Radish is also used for medicinal or food purposes using different parts of the radish plant including roots, leaves, sprouts, or oil from seeds (Shukla *et al*., 2012; CGIAR, n.d.).

1.3.1 Phenology

Radish can be cultivated as an annual or biennial vegetable (Zaki *et al*., 2012). Radish seed crops mature in approximate 120 – 150 DAS depending on variety and climate. According to Hampton & Young (1988), there are four developmental phases of a radish seed crop, namely vegetative growth, stem elongation, flowering and pod development (Table 1.4). However, Meier (2001) divided it into eight phases; germination, root development, leaf development, harvestable vegetative parts development, inflorescence emergence, flowering, fruit ripening, seed ripening and senescence. On the other hand, Malik (2009) described radish growth by five stages. They were cotyledon, '6 to 8' leaf, bolting, 50% flowering and 50% silique formation phases.

Radish is cross-pollinated by bees and other insects (CABI, 2018). There are two colours of radish flowers namely pink and white (Fig. 1.7) (CABI, 2018). When 60 - 70% of the seed pods have turned from green to brown (Fig. 1.8 A, B, C) and lose their fleshy appearance, becoming peppery thin and light, and when the moisture content of the seeds (Fig. 1.8D) is under 10%, the seeds are ready to be harvested (Hampton & Young, 1988; Nery *et al*., 2014).

Table 1.4. Phenology of radish seed plants in Palmerton North, New Zealand (Hampton & Young, 1988).

Weeks after sowing	Growth stage	Comments
1	Emergence	
3	Young rosette	First true leaf >5mm.
4	Rosette	The rapid development of 8-11 leaves.
5	Reproductive rosette	Growing point visibly reproductive.
7	Main stem elongatioin	Rapid stem growth to 20-30cm. Axillary shoots not diverged or elongated.
8	First anthesis	First flowers on terminal raceme of main stem open. Rapid elongation of the main stem and axillary branches continues.
11	Peak anthesis	Flower opening occurs acropetally along a raceme and basipetally from the terminal raceme.
14	Anthesis complete	Petal fall on lowermost axillary racemes. Rosette leaves sensesced.
16	Pods reach maximum size	Seed full-sized but mainly liquid endosperm tissue, translucent testae.
20	Harvest ripeness	Seed moisture content 10-12%.

Figure 1.7. Flower of radish seed crop: white (A) and pinky (B). Photos by author.

Figure 1.8. Pods (A, B, C) and seeds (D) in a radish seed crop. Photos by author.

1.3.2 Radish seed production in New Zealand

In New Zealand, vegetable seeds are produced for both export and domestic use (Millner & Roskruge, 2013). Seed production has been developed in recent years to supply Northern Hemisphere seed companies (Millner & Roskruge, 2013). The climate conditions in New Zealand are suitable for not only radish but also other vegetable seed production (Hampton *et al*., 2012).

New Zealand farmers grow seed crops on 40,000 hectares of land and produce 40,000 tonnes of seed annually (Scott, 2017). According to NZ Horticulture (2018), vegetable seed exports earned $92.4 million in 2018, up 33% on that of 2017 ($64.4 million). Vegetable seeds were exported to over 60 world markets in 2018. Major export markets were the Netherlands ($50.9 million), Korea ($7.6 million), the United States ($6.5 million), Japan ($6.3 million), Vietnam ($3.2 million), African countries ($2.4 million) and Australia ($2.3 million) (NZ Horticulture, 2018). The Netherlands is the largest market for carrot and radish seed and exports of vegetable seed to Asian countries have increased in recent years (Scott, 2017).

Among them, radish seed export was worth $23.9, $28.5, $22.8 and $25.1 million in 2015, 2016, 2017 and 2018, respectively (NZ Horticulture, 2017; NZ Horticulture, 2018). Continental Europe and Asia are the highest consumption markets of radish seeds, with $13 and $10 million, respectively in 2018 (NZ Horticulture, 2018). South Pacific Seeds NZ Ltd. (SPS) is one of the biggest companies producing radish seeds for export (Lee, 2018).

The radish seed crop was first grown commercially in New Zealand in the 1982-1983 season in the Ashburton area (Hampton & Young, 1988). Radish seed production is now widespread in the Canterbury plains for export purposes (McKay, pers. comm., 2019). Many trials have been carried out to examine the effects of various factors on seed quality, seed yield and pathogens of radish seed crops (Hampton & Young, 1988; FAR, 2016; FAR, 2017; Braithwaite *et al*., 2018; Lee, 2018; Khan, 2019).

1.3.3 Radish cultivation

The three popular radish seed varieties grown in New Zealand for export are Round Red, French Breakfast and Daikon (Taylor, pers. comm., 2019).

Radish seed crops can be grown using the root to seed or seed to seed method (George, 2009). However, the seed to seed method is used in New Zealand for radish seed production (Khan, 2019).

Radish seed requires a temperature from 20^{o}C to 30^{o}C for 4-10 days to germinate. However, a range of temperatures between 5 and 35^{o}C is suitable for radish development. The juvenile and flowering periods need vernalisation (CABI, 2018) at temperatures of 5 - 10^{o}C (Engelen-Eigles & Erwin, 1997).

Moreover, in hybrid radish seed production, the growers must follow strict isolation rules with 2000 metres from any other radish of the same variety, and 3500 metres from other radish seed production (McKay, pers. comm., 2019). Also, the radish seed production field must have been free of residual *Brassica* seeds for 7-15 years (McKay, pers. comm., 2019).

The radish seed crop requires soil which is well-drained, light, sandy loam or silt loam, deep and high organic matter with pH 6–6.5. Heavy soils might bring about misshapen roots (CABI, 2018; McKay, pers. comm., 2019).

Radish sowing rates depend on radish variety and hybrid production method. Hampton & Young (1988) investigated crop manipulation with a sowing rate of 5kg and 10kg per hectare

and the growth retardant paclobutrazol (PP333). Plots sown at 10 kg seed per hectare, in either 30 cm or 15 cm row spacing had a greater seed yield (121 g/m^2) than other treatments because more pods were retained per plant. Seeds sown at 10 kg per hectare produced the best potential seed yield per m^2, whereas those sown at 5 kg per hectare had the highest seed yield per plant.

New Zealand growers have been using radish seed treatments with fungicides and pesticides before sowing to reduce soilborne and seedborne pathogen attack (McKay, pers. comm., 2019). Seeds are sown at a depth of 8mm for small seeds and 15mm for larger seeds under dry conditions. Precision drilling is required for the hybrid Round Red radish variety (McKay, pers. comm., 2019). The hybrid Round Red radish variety requires a distance between plants and rows of 10cm and 50cm, respectively (Khan, 2019). The ratio is 2:6 rows of male and female lines for hybrid radish seed production (Khan, 2019). Sowing is between 25th August and 15th September, and harvesting between March and April of the following year (Khan, 2019; McKay, pers. comm., 2019).

Beehives are placed in and around the field whenever sufficient flowers on the crops appear to make sure of good cross-pollination. The removal of male beds is a requirement for hybrid radish seed production when the female has finished flowering (McKay, pers. comm., 2019).

1.3.4 Diseases

Soilborne and seedborne pathogens are threats to radish seed crop production in the world. They are *A. candida, Alternaria brassicae, Alternaria brassicicola, Alternaria raphani syn. A. matthiolae, Aphanomyces raphanin, Colletotrichum higginsianum, Fusarium oxysporum* f. sp. *raphanin, Gibberella avenacea, Leptosphaeria maculans, Peronospora parasitica, Plasmodiophora brassicae, Rhizoctonia solani, Xanthomonas campestris* pv. *raphanin, Xanthomonas oryzae* pv. *oryzae* pathogens (Lee, 2018). In New Zealand, *P. parasitica*, *A. brassicae*, *R. solani, Sclerotinia sclerotiorum* and *A. candida* are the main pathogens in radish (FAR, 2016; 2017; Lee, 2018; Taylor, pers. comm., 2019). Among them white blister disease on *Brassica* crops was first reported in *B. oleracea* (cabbage) in 1906, *B. oleracea* (cauliflower and Brussels sprouts) in 1922 (Hill, 1979; Pennycook, 1989), *C. bursa-pastoris* in 1924 (Baker, 1955), and radish in 1945 (Baker, 1955; Boesewinkel, 1977). At that time it was not considered an important pathogen for radish, but in 1996 when SPS started to grow radish seed crops, *A. candida* caused 95% yield losses (McKay, pers. comm., 2019). Since then there has been a

rapid spread not only in radish but also other brassica seed crops (Millner *et al*., 2013). This coupled with no effective control measure (FAR, 2016) has meant that in radish, for example, seed yield losses caused by white blister of over 50% have been recorded, a loss in monetary terms of nearly $6,000 per hectare (McKay, pers. comm., 2019).

The Foundation for Arable Research (FAR) has cooperated with farmers and pathologists to evaluate chemical control of white blister disease on radish seed crops (FAR, 2016; 2017; Braithwaite *et al*., 2018). Although no fungicide is registered in New Zealand for control of *A. candida*, Ridomil Gold MZ WG has shown promise (FAR, 2016). FAR (2016) conducted a field trial with mono-fungicide applications and one combination of fungicides (Seguris Flexi and Coment). Ridomil Gold MZ WG at 3 kg/ha, Pristine at 400 g/ha and Amistar at 1 L/ha as well as Seguris Flexi plus Coment increased seed yield, although only Pristine and Amistar reduced white blister infection. In the 2016-2017 season, FAR (2017) evaluated the presence of oospores on the seeds and the effects of single, combination and rotation of fungicides on seed yield and disease. The results showed the number of oospores on the radish seeds ranged from 0 to 395 oospores per gram of seed. Pristine at 1.5 L/ha produced a higher seed yield than Ridomil Gold MZ WG at 2.5 L/ha, a mix of Seguris Flexi plus Amistar and rotation of Amistar followed by Ridomil Gold MZ WG plus Amistar. Braithwaite *et al*. (2018) also reported that Metalaxyl-m plus Mancozeb, Azoxystrobin, Boscalid plus Pyraclostrobin, and Cyazofamid fungicides improved seed yields by up to 67%, and reduced disease infection by up to 50%. However, multiple fungicide application leading to fungicide resistance is a concern, especially when *A. candida* has both asexual and sexual reproduction which can result in repeat infection many time during the crop season.

There are lots of papers about *A. candida* but most of these are for mustard, rapeseed, broccoli, Brussels sprouts, turnip, etc., with very few reported on radish. Variations in the severity of *A. candida* epidemics might change because of environmental changes or the emergence of races between hosts *Brassica* species (Kaur, 2013). For example, *A. candida* race 2 was found in B. *juncea* spp. in North America (Pound & Williams, 1963), but races 12 – 34 were found on these species in India (Verma *et al*., 1999; Gupta & Saharan, 2002). Petkowski *et al.* (2010) found *B. nigra* was susceptible to both *A. candida* race 7 and 9 in Australia. Furthermore, Petkowski *et al.* (2010) also confirmed *A. candida* caused white blister disease on broccoli, black mustard, broccolini, Brussels sprout, cauliflower, oilseed rape and turnip rape in Australia which differs on *B. oleracea* and *B. rapa* from Europe. Race 1 has been

confirmed in radish in North America (Table 1.1) (Pound & Williams, 1963), but it is not known if this is also the race on radish in New Zealand. Minchinton *et al.* (2005) as cited in Kaur (2013) reported the presence of *A. candida* races 1, 2V, 3, 4, 5, 6, 7 and 9 in New Zealand. Kaur (2013) reported *A. candida* race 7 in *B. rapa* and race 9 in *B. oleracea* in New Zealand. However, different *A. candida* races could infect different varieties. For example, Yadav & Sharma (2004) found 21 cultivars of *B. juncea* were immune to *A. candida* but on *B. juncea* cv RL 1359, Kranti, RH 30, EC 182925 DVS 7-3-1, *A. candida* races 14-34 were detected (Jat, 1999; Gupta & Saharan, 2002). However, data about *A. candida* active races or resistant sources in radish and other *Brassica* species in New Zealand has not been reported as yet.

1.4 Aims and Objectives

The work undertaken in this study was to extend the current knowledge of *A. candida* on radish in New Zealand. First, there are no reports about the effectiveness of using seed treatments for control of *A. candida* in radish in New Zealand, although a fungicide seed treatment is routinely used in radish seed production by the industry. Second, non-chemical control methods to manage the white blister disease in radish have not been investigated. Third, the oomycete group includes pathogens now resistant to some fungicides, but whether this includes *A. candida* is not known. Thus, even though fungicide field trials were undertaken during previous years, but more research about fungicides is required, particularly different product application rates and time of applications. Finally, the transmission of *A. candida* in radish is not fully understood.

The aims of this study were to identify the transmission sources for *A. candida* to find effective methods for control of *A. candida* in radish and to determine the impact of the pathogen on seed yield and quality.

According to those questions, the research objectives were as follows:

1. To assess the ability of different seed treatment methods to control *A. candida*.

2. To evaluate the effects of foliar fungicides on white blister disease, radish seed yield and quality.

3. To determine whether (i) *A. candida* can infect the host internal structures and may survive within the plant and (ii) *A. candida* can be transmitted vertically via seeds.

Chapter 2

The ability of seed treatments to control *Albugo candida*

2.1 Introduction

Albugo candida is a seedborne pathogen (Petrie, 1975; Verma & Petrie, 1980; Jacobson *et al*., 1998; Minchinton, 2007). Therefore, seed treatment could become an important method of eradicating or reducing the pathogen. Fungicide seed treatments with metalaxyl and mancozeb have been used and reported to be effective in radish and other *Brassica* crops (Verma & Petrie, 1979; Stone *et al*., 1987; Saharan *et al*., 2014). However, the continuous chemical application could cause some negative effects such as the development of pathogen resistance. Non-chemical alternatives to fungicide seed treatment have been developed and two methods are hot water seed treatment (Williams, 1967; Nega *et al*., 2003; Groot *et al*., 2006; Toporek & Hudelson, 2017;) and seed coating using BCAs (Spadaro *et al*., 2017; Lee, 2018).

Trichoderma spp. applied as a seed treatment have provided control of soilborne and seedborne pathogens such as *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* spp. in different crops (Bennett & Whipps, 2008; Zhang *et al*., 2010; Wharton *et al*., 2012; Lee, 2018). However, there is a lack of research about the treatment of seeds to control *A. candida* by using heat and BCAs. Thus, the purpose of this study was to evaluate the effects of several seed treatment methods on white blister disease development in plants grown from infected seeds.

2.2 Materials and methods

Two experiments were carried out at Lincoln University. Seeds harvested from an *A. candida* infected radish seed crop in the 2017-2018 season were used. Five grams of radish seeds were used for each seed treatment (Appendix A).

The experiments were set up at different times in two glasshouses (Fig. 2.1). For experiment 1, non-graded seeds (i.e. small and shrivelled seeds had not been removed) were used. Plants were watered from the base only (into trays) to avoid oospore movement up the plant by water splash. For experiment 2, graded (2.6-3mm) seeds were used. Plants were watered by overhead spray. Experiment 1 (Appendix B) was from 5th November 2018 to 29th February 2019. By the end of the experiment, all plants were flowering and about 70% had immature

pods (Fig. 2.2). Experiment 2 (Appendix C) was from 19th December 2018 to 1st May 2019 by which stage 10% of plants had started to flower.

Each experiment included 10 seed treatments: two controls (untreated seeds), three heat seed treatments, two BCA seed treatments and three chemical seed treatments (Table 2.1, 2.2).

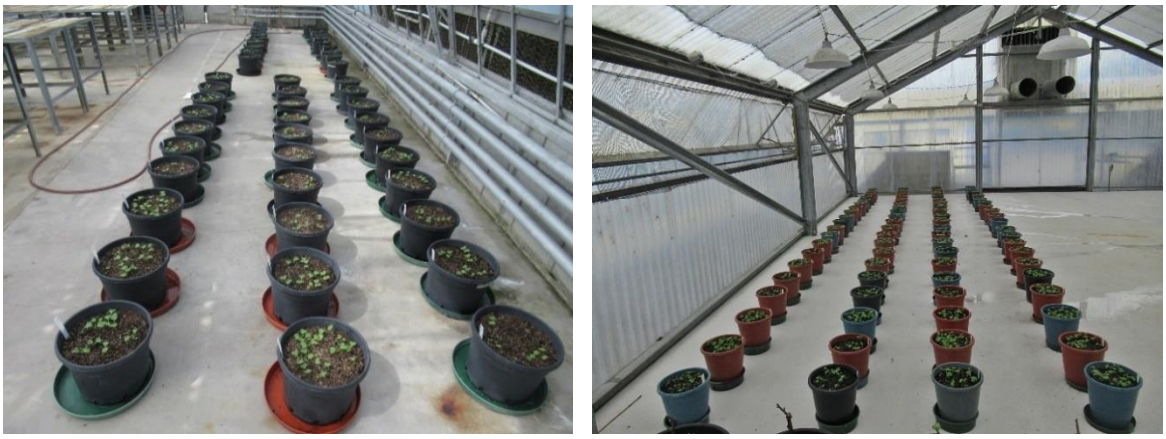


Figure 2.1. Experiments in the glasshouses: experiment 1 (left), experiment 2 (right). Photos by author.

Figure 2.2. Immature pods in experiment 1. Photos by author.

The purpose of this study was to determine effective seed treatment methods for controlling *A. candida* compared to the control. There were eight seed treatments to be compared to the control. For the most efficient usage of the experimental resources (like a fixed number of plots), statistical theory has shown that the control treatment should be replicated √8 = 2.8 times as heavily as each other treatment (Scheffé, 1959). We therefore included two control plots in each block in both experiments.

There are no reports about physical and BCA seed treatments to control *A. candida* in radish. However, hot water seed treatment at 50°C from 15 to 25 minutes has been reported as an

effective method to control some seedborne pathogens of *Brassica* species (Nega *et al*., 2003; Toporek & Hudelson, 2017) such as *Alternaria* spp. and *Phoma lingam* (Holtzhausen, 1978). Therefore, in this study, radish seeds were hot water treated at 50^{o}C for 15, 20 and 25 mins.

Trichoderma atroviride LU132 and LU140 are strains currently being used in research at the Bio-Protection Research Centre, New Zealand. *Trichoderma atroviride* LU132 has been used to control *Rhizoctonia solani* in radish (Lee, 2018) as well as control *Gaeumannomyces graminis* var. *tritici* in grasses (Umar, 2018).

Metalaxyl has been reported to reduce *A. candida* significantly when used as a seed treatment and a foliar fungicide (Verma & Petrie, 1979; Bhargava *et al*., 1997, Minchinton *et al*., 2004; Saharan *et al*., 2014). Metalaxyl currently is used to treat all radish seeds at SPS before sowing (McKay, pers. com., 2019). The combination of metalaxyl and mancozeb as a foliar fungicide has been effective for controlling *A. candida* in Australia (Minchinton, 2004) and New Zealand (Braithwaite *et al*., 2018). Thiram and Iprodione seed treatments have been used for control of *Alternaria* spp. in radish seeds in South Africa (Holtzhausen, 1978) and in Bangladesh (Islam *et al*., 2007), respectively.

Table 2.1. List of seed treatments in the experiments.

No.	Seed treatment [1]
1	Control
2	Control
3	Hot water 50^{o}C for 15 minutes
4	Hot water 50^{o}C for 20 minutes
5	Hot water 50^{o}C for 25 minutes
6	*Trichoderma atroviride* LU132 (10^7 CFU/ gram of seed)
7	*Trichoderma atroviride* LU140 (10^7 CFU/ gram of seed)
8	Metalaxyl and mancozeb (10mg Ridomil Gold MZ WG plus 500µL deionised water)
9	Thiram (10mg thiram plus 500µL deionised water)
10	Iprodione (10mg iprodione plus 500µL deionised water)

[1] *application rates for the fungicides are for the product.*

Table 2.2. Active ingredients for the chemical seed treatments in the experiments.

Fungicide treatment	Active ingredient
Ridomil Gold MZ WG	Metalaxyl-M (40 g/kg) plus mancozeb (640 g/kg)
Thiram	Thiram (800 g/kg)
Iprodione	Iprodione (500 g/kg)

- Heat seed treatments: radish seeds were treated at 50^{o}C for 15, 20 and 25 minutes (Table 2.1). First, seeds were wrapped in a permeable cloth and pre-warmed at 38°C in a water bath for ten minutes. After that, seeds were transferred to another water bath heated to 50^{o}C for 15, 20 or 25 minutes. Then seeds were then placed in cold tap water for five minutes to quickly end the heat treatment. Finally, seeds were spread out on a paper towel to air dry (Holtzhausen, 1978; Nega *et al*., 2003; Toporek & Hudelson, 2017).

- BCA seed treatment: radish seeds were treated with two strains of *T. atroviride* LU132 and LU140 obtained from the Bio-Protection Research Centre (Table 2.1). The seed was coated with conidia (10^7 CFU/ per gram of seed) enclosed in a commercial polymer (Umar, 2018).

- Chemical seed treatment: radish seeds were coated by hand with Metalaxyl and mancozeb (Ridomil Gold MZ WG, Syngenta New Zealand) (Bhargava *et al*., 1997; Rathi & Singh, 2009; Braithwaite *et al*., 2018), thiram (Thiram fungus control, Kiwicare) (Holtzhausen, 1978; Dange *et al*., 2003), and Iprodione fungicides (Roval WP, Arysta LifeScience) (Islam *et al*., 2007) at 10mg chemical in 500µl deionised water for each treatment (Table 2.1, 2.2).

The seed treatments were applied before sowing into Lincoln University's standard 3-4 months potting mix. It is comprised of 800L bark and 100L pumice in 8:1 ratio and enriched with 3000g osmocote extract 16-3.9-10 (3-4 months), 1000g horticultural lime and 1000g hydraflo in a ratio of 3:1:1 for a volume of 1m^3. Both experiments were arranged in a randomized complete block design (RCBD) with 8 replicates per treatment. Twenty seeds were sown in individual 10 litre pots. Seedling emergence assessment began at four DAS. All the plants were kept to determine the ability of seed treatments to control the pathogen until 116 DAS for experiment 1 and 160 DAS for experiment 2. The glasshouse temperatures were recorded every week. The record showed an average monthly temperature of 28^{o}C during December and January and 27^{o}C during February and March throughout the experimental

period (Fig. 2.3). Temperatures increased by around 5°C from November to December and fell considerably (5°C) from April to May. During the experiments in the glasshouses, insect attack occurred (Apendix D) and Pilarking® 220 g/litre (imidacloprid in the form of a soluble concentrate), Methylene chloride 760g/litre and Success Naturalyte 120 g/litre (spinosad in the form of a suspension concentrate) insecticides were sprayed on plants on 28th January 2019.

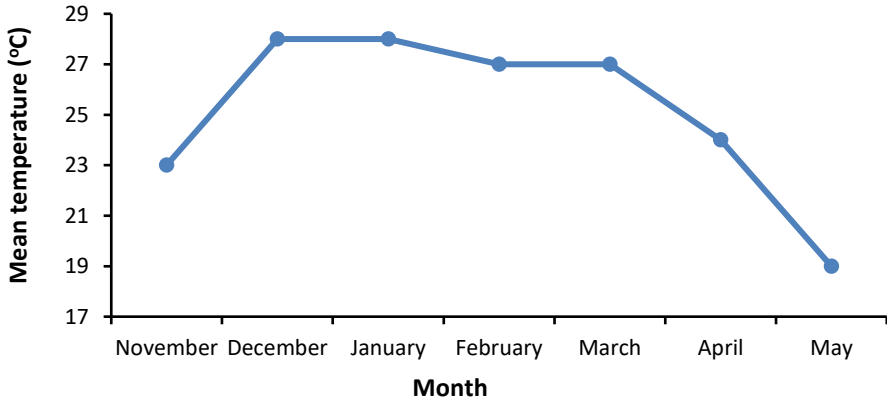


Figure 2.3. Mean temperatures in the glasshouses.

2.3 Assessments

2.3.1 Seedling emergence

The number of normal seedlings (ISTA, 2018) emerged (Fig. 2.4) in each pot were counted every two days from 4 DAS to 18 DAS.

Figure 2.4. Radish normal seedling emergence in a pot at 4 DAS. Photo by author.

2.3.2 Disease incidence

Disease incidence data were recorded once every week after the first *A. candida* sori were observed on one or more leaves as follows (Asif *et al*., 2017):

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants examined}} \times 100$$

2.3.3 Disease severity

Disease severity data were recorded at the same time as disease incidence by visual observation from the initiation of the first occurrence of disease symptoms until the leaves had become necrotic. The disease lesions were assessed on each leaf of a plant. Severity was rated on a 0-5 scale: 0= no lesions, 1= scattered lesions on the lower leaves, 2= low numbers of lesions on the upper leaves, 3= moderate number of lesions on the lower leaves, low level of lesions on the upper leaves, 4= moderate to high numbers of lesions on the lower leaves, greater than 200 per leaf and moderate number of lesions on the upper leaves, 5= moderate to heavy number of lesions over the whole plant, greater than 200 per leaf (Braithwaite *et al.,* 2018). Then, the disease severity was calculated using the following formula (Wheeler, 1969; Sandhu *et al*., 2015):

$$\text{Disease severity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants examined x maximum disease grade (5)}} \times 100$$

2.4 Statistical analysis

All data were analysed using an analysis of variance (ANOVA) for RCBD, using Genstat 19th edition (VSN International, Hemel Hempstead, UK). The unrestricted least significant difference (LSD) procedure at 5% significance level was used to test the differences among treatments.

The average of the two control treatments for each experiment is presented in the results.

The relationship between disease incidence and disease severity in each experiment was determined by regression analysis.

2.5 Results

2.5.1 Seedling emergence

Table 2.3. Effect of seed treatments on final seedling emergence percentage at 18 DAS.

Seed treatment	Experiment 1 (%)	Experiment 2 (%)
Control	52.8 [a]	83.4 [d]
Hot water 50°C/ 15 minutes	50.0 [a]	66.3 [abc]
Hot water 50°C/ 20 minutes	57.5 [a]	65.6 [abc]
Hot water 50°C/ 25 minutes	45.6 [a]	60.0 [a]
Trichoderma atroviride LU132	47.5 [a]	68.1 [abc]
Trichoderma atroviride LU140	53.1 [a]	69.4 [bc]
Metalaxyl and mancozeb	51.9 [a]	68.8 [abc]
Thiram	43.8 [a]	74.4 [c]
Iprodione	51.9 [a]	61.3 [ab]
LSD (5%) for		
Ctrl vs Another Trt	12.5	8.1
One Trt vs Another Trt	14.4	9.3

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

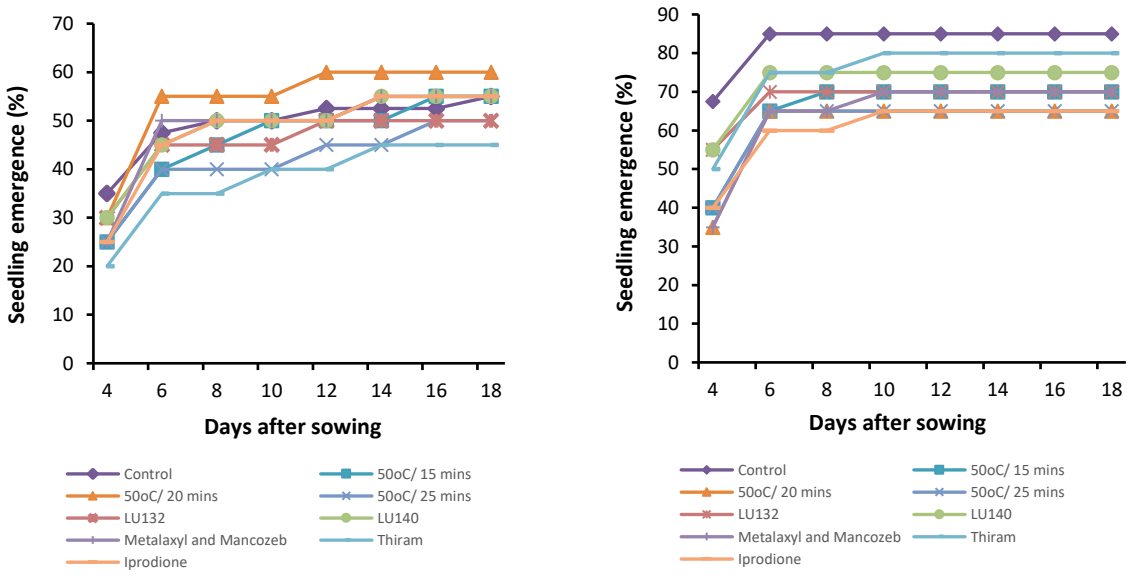


Figure 2.5. Effect of seed treatments on radish seedling emergence for experiment 1 (left) and experiment 2 (right).

The final emergence of experiment 1 was lower than in experiment 2 (Table 2.3). There were no significant differences found among seed treatments in experiment 1. However, in experiment 2, the control treatment had a significantly higher seedling emergence ($P < 0.05$) compared to the other treatments (Table 2.3). Among seed treatments, Thiram had a higher

emergence than hot water 50°C/ 25 mins and Iprodione, and hot water 50°C/ 25 mins had a lower emergence than *T. atroviride* LU140. Seedlings in experiment 1 took a longer time to reach maximum emergence (16 days) than in experiment 2, which only required 10 days (Fig. 2.5).

2.5.2 Disease assessment

2.5.2.1. Days to first symptom appearance

There were no differences among treatments in days to first symptom appearance in either experiment (Table 2.4). The first symptoms were observed on the leaves in experiment 1 at 71 DAS, later than in experiment 2 which was 44 DAS (Table 2.4). Within 8 days (from 71 DAS to 79 DAS) and 6 days from (44 DAS to 50 DAS) all first symptoms for all treatments were recorded in experiment 1 and experiment 2, respectively.

Table 2.4. Effect of seed treatments on the number of days to the first symptom appearance.

Seed treatment	Experiment 1 (DAS)	Experiment 2 (DAS)
Control	71.8 [a]	47.9 [a]
Hot water 50°C/ 15 minutes	70.8 [a]	46.1 [a]
Hot water 50°C/ 20 minutes	71.4 [a]	44.3 [a]
Hot water 50°C/ 25 minutes	74.4 [a]	49.5 [a]
Trichoderma atroviride LU132	73.5 [a]	44.6 [a]
Trichoderma atroviride LU140	75.5 [a]	47.4 [a]
Metalaxyl and mancozeb	76.8 [a]	48.5 [a]
Thiram	76.8 [a]	46.0 [a]
Iprodione	78.8 [a]	45.9 [a]
LSD (5%) for		
Ctrl vs Another Trt	8.1	5.5
One Trt vs Another Trt	9.3	6.4

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

2.5.2.2. Disease incidence and disease severity

Table 2.5. Effect of seed treatments on disease incidence and disease severity for experiment 1 at 59, 73 and 116 DAS.

Seed treatment	Disease incidence (%)			Disease severity (%)		
	59 DAS	73 DAS	116 DAS	59 DAS	73 DAS	116 DAS
Control	1.9 [a]	14.5 [ab]	37.0 [a]	0.4 [a]	4.1 [a]	20.3 [ab]
Hot water 50°C/ 15 minutes	2.3 [a]	26.9 [b]	44.3 [ab]	0.5 [a]	5.9 [a]	22.9 [ab]
Hot water 50°C/ 20 minutes	0.7 [a]	18.6 [ab]	40.7 [ab]	0.1 [a]	4.8 [a]	22.4 [ab]
Hot water 50°C/ 25 minutes	0.0 [a]	13.5 [ab]	35.0 [a]	0.0 [a]	4.2 [a]	21.0 [ab]
Trichoderma atroviride LU132	0.0 [a]	15.4 [ab]	41.6 [ab]	0.0 [a]	4.0 [a]	21.3 [ab]
Trichoderma atroviride LU140	2.2 [a]	18.5 [ab]	42.4 [ab]	0.4 [a]	5.2 [a]	21.3 [ab]
Metalaxyl and mancozeb	0.0 [a]	11.6 [ab]	33.0 [a]	0.0 [a]	2.3 [a]	17.8 [a]
Thiram	0.0 [a]	16.7 [ab]	55.3 [b]	0.0 [a]	3.4 [a]	30.0 [b]
Iprodione	0.0 [a]	9.3 [a]	34.8 [a]	0.0 [a]	1.9 [a]	18.5 [ab]
LSD (5%) for						
Ctrl vs Another Trt	2.7	14.6	16.5	0.5	4.7	10.1
One Trt vs Another Trt	3.1	16.9	19.1	0.6	5.4	11.6

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Table 2.6. Effect of seed treatments on disease incidence and disease severity for experiment 2 at 42, 69 and 160 DAS.

Seed treatment	Disease incidence (%)			Disease severity (%)		
	42 DAS	69 DAS	160 DAS	42 DAS	69 DAS	160 DAS
Control	1.9 [a]	91.9 [a]	100.0	0.4 [a]	30.4 [a]	62.8 [a]
Hot water 50°C/ 15 minutes	1.3 [a]	83.8 [a]	100.0	0.3 [a]	26.8 [a]	62.0 [a]
Hot water 50°C/ 20 minutes	2.5 [a]	87.5 [a]	100.0	0.5 [a]	30.5 [a]	62.0 [a]
Hot water 50°C/ 25 minutes	2.5 [a]	85.0 [a]	100.0	0.5 [a]	27.3 [a]	62.3 [a]
Trichoderma atroviride LU132	2.5 [a]	87.5 [a]	100.0	0.5 [a]	28.0 [a]	62.8 [a]
Trichoderma atroviride LU140	2.5 [a]	91.3 [a]	100.0	0.3 [a]	30.3 [a]	64.3 [a]
Metalaxyl and mancozeb	1.3 [a]	86.3 [a]	100.0	0.3 [a]	29.3 [a]	63.0 [a]
Thiram	1.3 [a]	90.0 [a]	100.0	0.3 [a]	29.0 [a]	63.5 [a]
Iprodione	3.8 [a]	91.3 [a]	100.0	0.8 [a]	67.3 [a]	63.0 [a]
LSD (5%) for						
Ctrl vs Another Trt	3.5	11.2	-	0.7	29.1	3.3
One Trt vs Another Trt	4.0	12.9	-	0.8	33.6	3.9

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

In both experiments, disease incidence and disease severity for all the treatments did not differ from that of the control (Table 2.5, 2.6), except for Thiram at 116 DAS in experiment 1 for which disease incidence was greater than that in the control (P<0.05) (Table 2.5).

Disease incidence in experiment 1 fluctuated from treatment to treatment more than in experiment 2 (Fig. 2.6). In experiment 1, disease incidence was variable among treatments from 64 DAS to 87 DAS. In experiment 2, disease incidence rose up gradually to reach a peak at 83 DAS then did not change until 160 DAS (Fig. 2.6).

In experiment 1, disease severity increased slightly from 57 DAS to 116 DAS for all seed treatments (Fig. 2.7). In experiment 2, disease severity was the same for all seed treatments from 36 DAS to 48 DAS (Fig. 2.7), then fluctuated between 48 DAS and 97 DAS, before becoming stable at 160 DAS (Fig. 2.7).

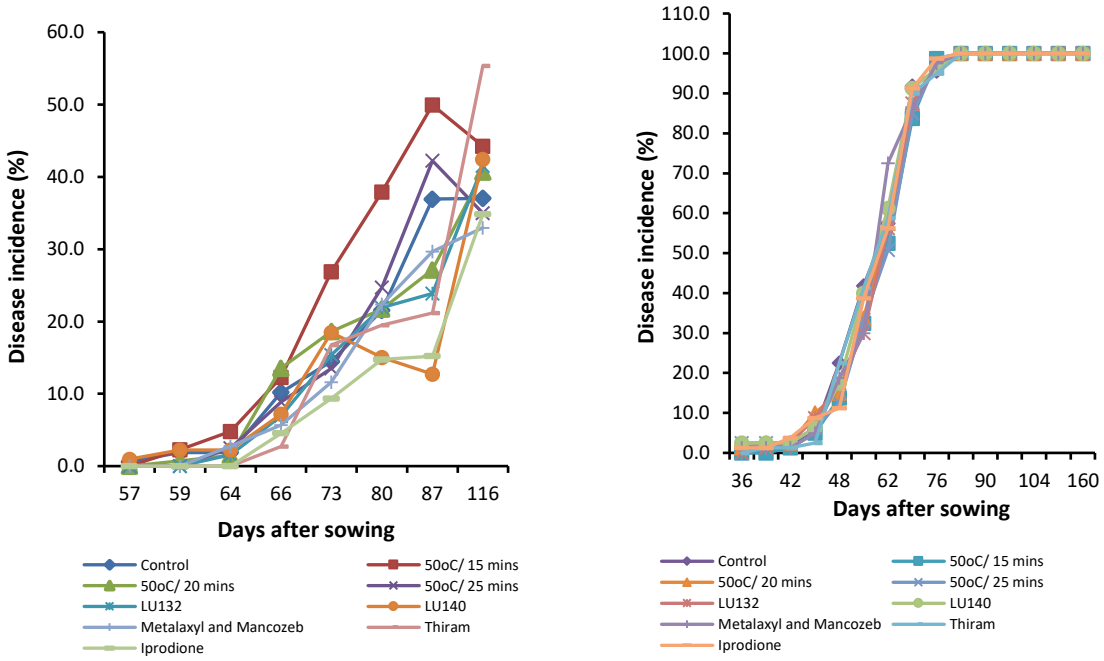


Figure 2.6. Effect of seed treatments on disease incidence for experiment 1 (left) and experiment 2 (right).

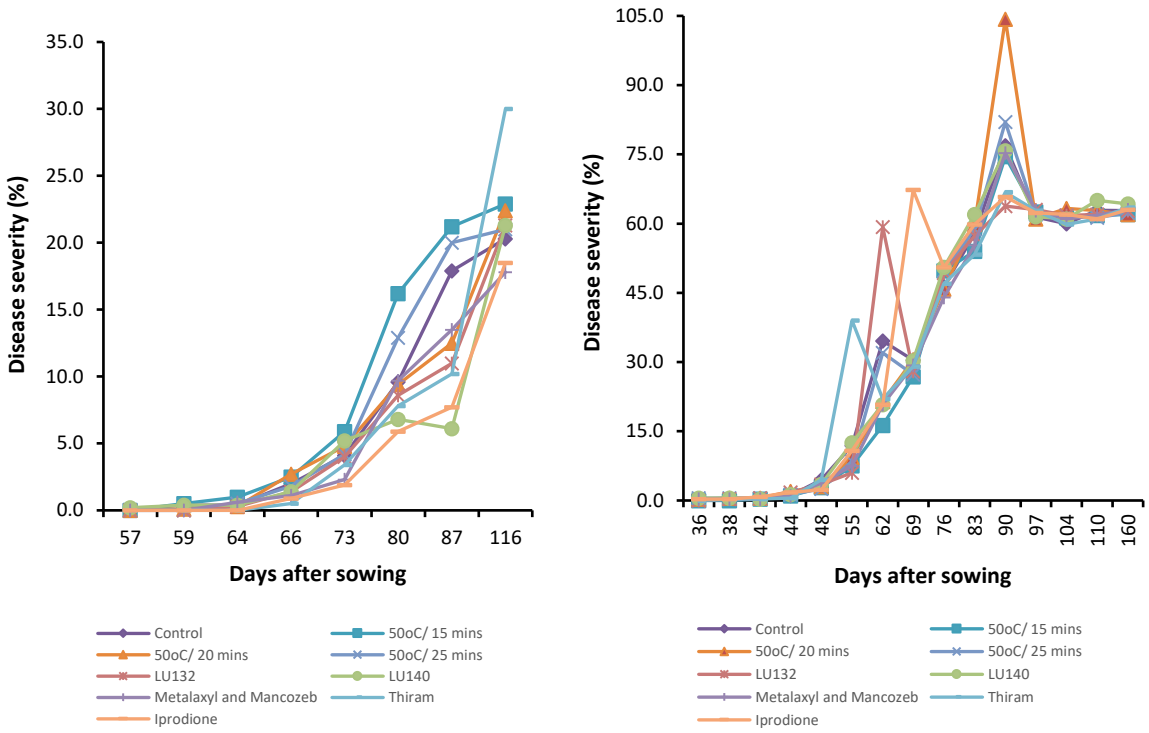


Figure 2.7. Effect of seed treatments on disease severity for experiment 1 (left) and experiment 2 (right).

2.6 Discussion

2.6.1 Seedling emergence

The higher emergence in the control in experiment 2 than in experiment 1 reflected the difference in seed quality. Seeds sown in experiment 2 had been graded to remove small and shrivelled seeds, which were still present in the seeds sown in experiment 1. The germination percentage of the two seed lots had not been assessed prior to sowing, but from the emergence recorded, seeds used in experiment 1 must have contained a higher number of dead seeds.

While seed treatments had no effect on emergence in experiment 1, they all significantly reduced emergence in experiment 2. This result was unexpected as seed treatments do not usually reduce seedling emergence (Heer, 1998; Salmon & Dumbleton, 2006). However, reduced emergence following seed treatment can occur if seed coat cracking allows access of the treatment to the embryo (Halmer, 2000). Whether this was the reason for the results recorded in experiment 2 was not determined.

2.6.2 Disease assessment

The incidence and severity of white blister disease did not differ among treatments within each experiment, but did differ between experiments, with the incidence and severity of the pathogen in experiment 2 being higher than in experiment 1. This was due to the different irrigation methods. Overhead irrigation allows splash – dispersal of the disease, while watering into the tray irrigation does not. Under the favourable conditions offered by free water, sporangia will germinate and release zoospores to cause infection (Liu, 1992). This step can occur following every watering during the experiment. Therefore, the white blister disease will spread. Moreover, water from the irrigation rose fell vertically, so that the zoospores spread horizontally (Gregory *et al*., 1959), from plant to plant, helping to ensure that all plants in the experiment became infected. Kadow & Anderson (1940), Lakra & Saharan (1989), Minchinton *et al*. (2004), Harvey (2010) and Arora *et al.* (2019) confirmed that the disease incidence of white blister increased significantly in radish and mustard crops if leaves were wet. Overhead irrigation has also been reported to increase the spread of various pathogens such as *Corynebacterium michiganense*, *Fusarium* spp., *Pseudomonas phaseolicola*, *Colletotrichum phomoides*, *P. infestans* and *Phytophthora* spp. in tomato, banana, bean, pea, potato and citrus (Rotem & Palti, 1969; Du Toit, 2004).

In this study, none of the seed treatments applied provided control of *A. candida*. Stone *et al.* (1987), Bhargava *et al*. (1997) and Minchinton *et al.* (2004) showed the most effective method for control of *A. candida* was a combination of metalaxyl seed treatment and foliar fungicides. Most of the research about the effectiveness of metalaxyl and manconzeb fungicides for control of *A. candida* has involved following by application for approximately three months (Verma & Petrie, 1979; Stone *et al*., 1987; Bhargava *et al.,* 1997; Rathi & Singh, 2009; Saharan *et al.,* 2014). Therefore, in the present study *A. candida* was able to spread because there were no foliar fungicide applications during the growing season. Valdes & Edgington (1983) and Liu (1992) reported that *A. candida* race 1 was not controlled by metalaxyl. If the race used in these glasshouse trials was race 1, this may explain why metalaxyl did not control the pathogen on seed, but does not explain why other treatments were also ineffective. Further research is required.

Chapter 3

Effects of foliar fungicides on white blister disease, seed yield and seed quality of radish

3.1 Introduction

The white blister is a common disease of the brassica family around the world. In mustard, it caused yield losses of up to 60% in India (Lakra & Saharan, 1989). In New Zealand, chemical control of the disease on radish has been investigated since 2013 (FAR, 2016). A non-registered fungicide Ridomil Gold® foliar showed promise, followed by Amistar and Pristine foliar fungicides (FAR, 2016, 2017). However, no fungicide gave complete control. The purpose of this study was to find the most effective fungicide treatment for control *A. candida* on radish by (i) comparing the effect of using one fungicide and a rotation of different fungicides applied at different stages of radish growth and (ii) the effect of fungicide application rate, and to determine their effects on seed yield and seed quality.

3.2 Materials and methods

The experiment was conducted in a commercial radish seed production field at Leeston, Canterbury (Fig. 3.1A). The grower controlled all inputs (fertiliser, herbicides and irrigation) except for foliar fungicide applications. The grower had a contract to produce hybrid radish seed for SPS. The soil in the paddock was a Temuka Silt Loam, which had a pH of 6. The radish variety was Round Red. Radish seeds had been treated with the insecticide Gaucho (12 ml/kg) and the fungicides Wakil (2 g/kg), Systiva (1.25 ml/kg) and Raxil Star (0.5 ml/kg) before sowing. The herbicides were also applied pre-planting. Radish seeds were sown on 19th September 2018. The sowing depth was 8mm. Seeds were sown using a precision air seed drill. The ratio of female and male rows was 6:2 (Fig. 3.1B). Male beds were removed at 144 DAS. Agrichemicals, fertilizer and irrigation were applied by the grower (Appendix F).

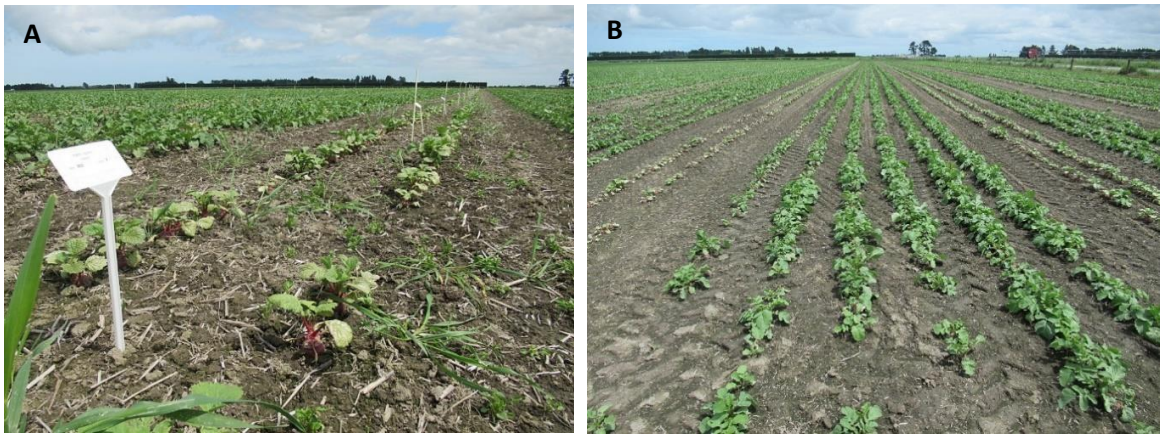


Figure 3.1. Field trial at Leeston, Canterbury, 2018 - 2019 season. Photos by author.

The radish field trial was organised and designed by FAR, laid out as a RCBD with 12 foliar fungicide treatments (Table 3.1, 3.2) and four replicates of each treatment. Each plot included two female beds (Fig. 3.2). Each bed was 2.5m in width and 3m in length. The distances between plants and rows were 10cm and 50cm, respectively (Fig. 3.2). Foliar fungicides were applied by FAR as per the schedule shown in Table 3.1. There were five applications during the season. The first application was at the 5-6 leaf stage on 22nd November 2018, followed by applications every 14 days after the first spray application (Table 3.1). Also, TriBase Blue (copper) bactericide and Lorsban 500ml insecticide were added to all spray treatments. Disease severity was determined every week from after the second foliar fungicide application until 3rd January 2019 using the method described in 2.3.3. Mean staghead lesion length per plant was assessed on 10th and 17th January 2019. Radish plants had lodged after that, so the disease assessment in the field trial could not carry on.

When there were sufficient flowers on the crop, two beehives per hectare were introduced into the field, placed to ensure good cross-pollination. When the pods began to fill and mature, they attracted birds to the field (Fig. 3.3A). Therefore, bird scarers were used to reduce pod damage caused by birds (Fig. 3.3B).

Six randomly selected radish plants per plot were removed from the field on 11th March 2019 (Fig. 3.4A). The cut plants then were left at the Field Research Centre, Lincoln University (Fig. 3.4B) to assess the white blister disease including mean staghead lesion length per plant, number of infected raceme tips, percentage of stems with symptoms, percentage of infected bulbs, percentage of infected pods and percentage of stagheads per plant.

Table 3.1. Foliar fungicides and product application rates in the radish seed field trial, 2018 -2019 season.

Application Date / Treatment	22/11/2018 (65 DAS)	07/12/2018 (80 DAS)	17/12/2018 (90 DAS)	07/1/2019 (111 DAS)	28/1/2019 (132 DAS)
1	Control (Nil)	Control (Nil)	Control (Nil)	Control (Nil)	Control (Nil)
2	2.5 kg/ha Ridomil Gold MZ WG	2.5 kg/ha Ridomil Gold MZ WG	2.5 kg/ha Ridomil Gold MZ WG	2.5 kg/ha Ridomil Gold MZ WG	2.5 kg/ha Ridomil Gold MZ WG
3	4 kg/ha Ridomil Gold MZ WG	4 kg/ha Ridomil Gold MZ WG	4 kg/ha Ridomil Gold MZ WG	4 kg/ha Ridomil Gold MZ WG	4 kg/ha Ridomil Gold MZ WG
4	2 L/ha Cobra	2 L/ha Cobra	2 L/ha Cobra	2 L/ha Cobra	2 L/ha Cobra
5	0.4 L/ha Metalaxyl-M	0.4 L/ha Metalaxyl-M	0.4 L/ha Metalaxyl-M	0.4 L/ha Metalaxyl-M	0.4 L/ha Metalaxyl-M
6	3.2 L/ha Max CL	3.2 L/ha Max CL	3.2 L/ha Max CL	3.2 L/ha Max CL	3.2 L/ha Max CL
7	5 L/ha Foschek + 0.4 L/ha Metalaxyl-M	5 L/ha Foschek + 0.4 L/ha Metalaxyl-M	5 L/ha Foschek + 0.4 L/ha Metalaxyl-M	5 L/ha Foschek + 0.4 L/ha Metalaxyl-M	5 L/ha Foschek + 0.4 L/ha Metalaxyl-M
8	350 ml/ha Zorvec	350 ml/ha Zorvec	350 ml/ha Zorvec	350 ml/ha Zorvec	350 ml/ha Zorvec
9	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb + 0.75 L/ha Amistar	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb + 0.6 L/ha Seguris Flexi	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb	2.5 kg/ha Ridomil Gold MZ WG + 400 g/ha Pristine	2.5 kg/ha Ridomil Gold MZ WG
10	2.5 kg/ha Ridomil Gold MZ WG + 0.75 L/ha Amistar	2.5 kg/ha Ridomil Gold MZ WG + 0.6 L/ha Seguris Flexi	2.5 kg/ha Ridomil Gold MZ WG	0.2 l/ha Ranman + 2.1 kg/ha Mancozeb + 400 g/ha Pristine	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb
11	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb + 0.6 L/ha Seguris Flexi	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb + 0.75 L/ha Amistar	2.5 kg/ha Ridomil Gold MZ WG + 400 g/ha Pristine	2.5 kg/ha Ridomil Gold MZ WG
12	2.5 kg/ha Ridomil Gold MZ WG	2.5 kg/ha Ridomil Gold MZ WG + 0.6 L/ha Seguris Flexi	2.5 kg/ha Ridomil Gold MZ WG + 0.75 L/ha Amistar	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb + 400 g/ha Pristine	0.2 L/ha Ranman + 2.1 kg/ha Mancozeb

Table 3.2. Active ingredients of foliar fungicides used in the field trial.

Fungicide treatment	Active ingredient
Amistar	Azoxystrobin (250 g/L)
Cobra	Dimethomorph (90 g/L) plus Chlorothalonil (450 g/L)
Ranman	Cyazofamid (400 g/L)
Ridomil Gold MZ WG	Metalaxyl-M (40 g/kg) plus Mancozeb (640 g/kg)
Max CL	Metalaxyl (50 g/L) plus Chlorothanonil (375 g/L)
Foschek	Phosphorous acid (400 g/L)
Mancozeb	Mancozeb (750 g/kg)
Zorvec	Oxathiapiprolin (100 g/L)
Metalaxyl -M	Metalaxyl (40 g/kg)
TriBase Blue	Copper Sulphates (190 g/L)
Pristine	Boscalid (252 g/kg) plus Pyraclostrobin (128 g/kg)
Seguris Flexi	Isopyrazam (125 g/L)

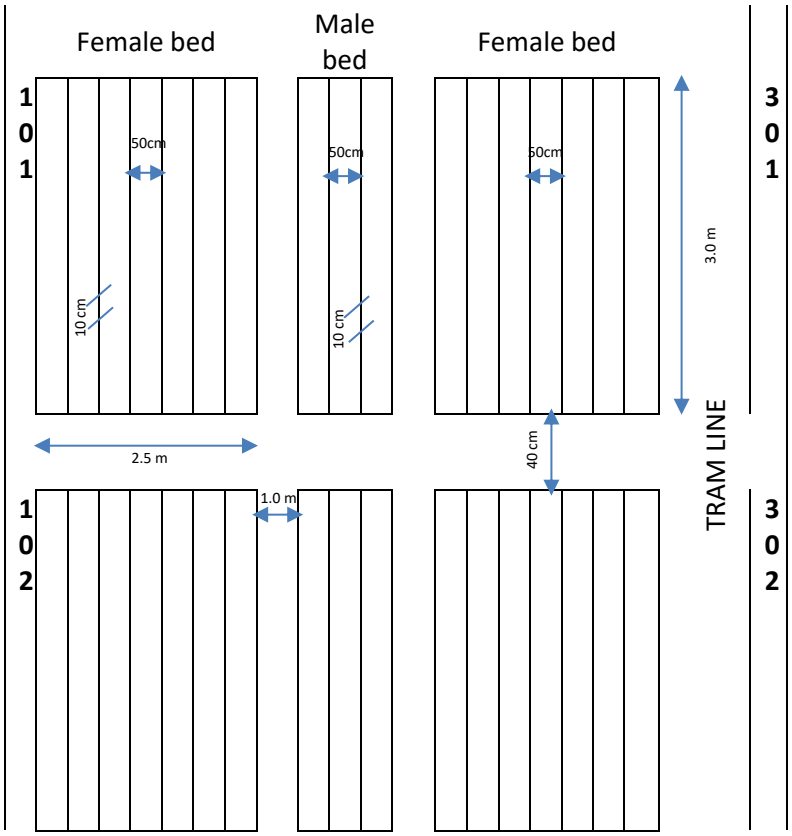


Figure 3.2. Radish field trial layout at Leeston.

Figure 3.3. Pods damaged by birds (A) and a bird scarer (B) in the field trial. Photos by author.

Figure 3.4. Radish plant samples collected from the field trial on 11th March 2019. Photos by author.

Reglone desiccant was applied on 12th March 2019. Seeds were harvested on 1st April 2019 by a seed combine harvester (Sampo 2010 combine cut 2.1m wide cut) provided by New Zealand Arable (Fig. 3.5A). Seeds from all 48 plots were harvested and placed in individual bags which were tied with a knot including a tag (Fig. 3.5B, C, D). Seeds then were weighed and left under ambient conditions at the Field Research Centre, Lincoln University before being taken to SPS in Methven for primary seed cleaning on 30th April 2019. The radish seeds were threshed on a thresher machine made by SPS. It is called a Humbler. The Humbler is often used to brush

hairs or tails off seeds (Fig. 3.5E), but it is also an effective "threshing machine" for small lots of radish seeds or pods (McKay, pers. comm., 2019). It has a rotating centre shaft with protruding steel pegs. These steel pegs (on the rotating shaft) pass between other steel pegs that are attached to the outer casing. The pods were crushed between adjustable heavy steel rollers set at a gap wide enough to crack the pod but not cause damage to the seeds. After threshing, seeds were cleaned on a commercial air screen cleaner (Sutton). It was made by Mark Sutton Engineering Ltd., Christchurch, New Zealand (Fig. 3.5F). It is the basic machine of the seed cleaning process. It has two air suctions, one before the first screen and one after the last screen. These air suctions remove light material from the seeds. The light material is lifted in the air suction and the heavy seeds are not lifted. The machine also has a number of screens (thin metal sheets that have precise sized holes in them). The seeds can either pass through the holes or not. Therefore, 9 mm and 5 mm screens were used to separate seeds from the larger and smaller chaff or weed seeds (Khan, 2019). Seeds fell through the big hole screen (9 mm) and then they stayed on top of the small hole screen (5 mm). After that, the operator chose the desired seed size screens and inserted these screens into the machine from the largest at the top to the smallest at the bottom. Thus, seeds were separated into three categories <2.6 mm, 2.6-3 mm and >3 mm. Seeds from each size group then were weighed, placed in a zip-seal plastic bag and stored in moisture-proof plastic containers at a temperature of 5-8^{o}C at the Bio-Protection Research Centre, Lincoln University until seed yield, seed quality and seed health assessment.

Weather data throughout the growing season were collected from the Broadfield weather station (the National Institute of Water and Atmospheric Research, NIWA, 2019). The climate data included monthly rainfall, mean relative humidity, mean temperature, and maximum and minimum temperature from 19th September 2018 to 31st March 2019.

Figure 3.5. Radish seeds from harvesting (A, B, C, D) to primary cleaning (E, F). Photos by author.

3.3 Assessment

3.3.1 Disease assessment

Disease assessment was carried out in two periods of radish growth and development. First, white blister disease severity and mean staghead lesion length per plant were evaluated starting from the second foliar fungicide application and continuing until the first pods had formed and branches became mixed (Fig. 3.6). Plants at the edge of each plot were not assessed. The second assessment was two weeks before harvesting seeds. Six randomly selected radish plants per plot were removed from the field, avoiding the edges. White blister infection of bulbs was assessed for all six plants. Five randomly selected racemes per plant and ten randomly selected mature pods per plant were then used to evaluate white blister infection of inflorescences, stems, raceme tips and pods. Pod damage caused by birds as well as the number of seeds per pod of each plant were counted.

Figure 3.6. First pods formed and mixed branches in the field. Photo by author.

Disease assessment in the field:

- Disease severity (%): ten randomly selected true leaves per plot were assessed at 86, 93 and 100 DAS (Fig. 3.7A) using the method and formula described in 2.3.3.

- Mean staghead lesion length per plant (mm): when the racemes developed and 50% of flowers had opened, the mean staghead lesion length per plant was measured at 107 DAS and 114 DAS (Fig. 3.7C), using a ruler. Again, plants on the plot edge were not included.

$$\text{Mean staghead lesion length per plant (mm)} = \frac{\text{Sum of staghead lesion lengths (mm)}}{\text{Total number of plants examined}}$$

Disease assessment at Lincoln University:

- The number of raceme tips infected by *A. candida*: total number of infected tips were counted from five randomly selected racemes from each plot.

- Percentage of stems with symptoms caused by *A. candida* (Fig. 3.7D): percentage of stems with symptoms on the stem was evaluated by using a 0 - 100% disease rating scale, guided by Rolston (2019). The results showed the total percentage of infected whole stems of five randomly selected racemes of each plot.

- Mean percentage of bulbs infected by *A. candida* (Fig. 3.7E) was assessed by counting the number of infected bulbs from each plot, then calculated using the equation below:

$$\text{Mean bulb infection (\%)} = \frac{\text{Number of infected bulbs}}{\text{Total number of bulbs examined}} \times 100$$

- Mean Percentage of pods infected by *A. candida* (Fig. 3.7B) was assessed by the same method as for the percentage of bulbs infected:

$$\text{Mean pods infection (\%)} = \frac{\text{Number of infected pods}}{\text{Total number of pods examined}} \times 100$$

- Mean percentage of stagheads per plant (Fig. 3.7C) was calculated using the number of stagheads recorded per plant and the number of racemes per plant using the formula given below (Ram & Awasthi, 2018):

$$\text{Mean stagheads per plant (\%)} = \frac{\text{Number of stagheads recorded}}{\text{Total number of racemes examined}} \times 100$$

Figure 3.7. Infected samples of radish from the field trial: leaf (A), pods (B), staghead (C), inflorescence stems (D), bulb (E). Photos by author.

3.3.2 Seed yield and yield components

- Seed field dressed yield and seeds per plant yield were calculated based on the total seeds before cleaning, followed the equations below:

$$\text{Seed field dressed yield (g/m}^2\text{)} = \frac{\text{Seed field dressed weight per plot (g)}}{\text{Plot size (width x length) (m}^2\text{)}}$$

$$\text{Seed plant yield (g/plant)} = \frac{\text{Seed field dressed weight per plot (g)}}{\text{Number of plants per plot}}$$

- The number of seeds per pod: the six randomly selected radish plants per plot were removed from the field before harvesting, excluding the edges. Ten randomly selected mature pods per plant were threshed by hand to count the number of seeds per pod (Fig. 3.8). The results were the average of sixty pods per plot.

- Weight of seed size fraction: seeds per plot were divided into three size categories <2.6mm, 2.6-3mm and >3mm after cleaning at SPS. Each seed size fraction then was weighed. The results for each fraction were reported as the weight of seeds in each size category and percentage by weight of the total plot weight.

Figure 3.8. Counting seeds in pods. Photo by author.

3.3.3 Seed quality

After cleaning, seeds then were stored in a zip-seal plastic bag and placed in moisture-proof plastic containers at 5-8^{o}C at Bio-Protection Research Centre, Lincoln University before and during testing. Seed quality was determined using methods from the ISTA rules (ISTA, 2018) except for the conductivity test (see 3.3.3.5). The assessments were carried out at the Bio-Protection Research Centre's laboratories. Randomly selected pure seeds from each seed size fraction from each plot were used to test moisture, purity seed, thousand seed weight, germination (ISTA, 2018) and conductivity (Demir *et al*., 2012; Khan, 2019).

3.3.3.1. Seed moisture content (SMC) test

The moisture content of a seed sample is the loss in weight when it is dried and expressed as a percentage of the weight of the original sample (ISTA, 2018). For radish, the low constant temperature oven method was used (ISTA, 2018). All weights were to three decimal places. First, an empty aluminium container was weighed with its lids (M_1). Then, 4-5g randomly selected seeds from a sample were placed into the aluminium container, covered with its lid and weighed together (M_2). An oven (Contherm, Thermotec 2000) was set at 103^{o}C. After

weighing, the container with seeds was placed into the oven with its lid removed for 17 hours. The container was then removed from the oven and covered with its lid. The container then was placed to cool for 15 minutes in a desiccator. After cooling, the container was weighed with its contents (M_3). The SMC was calculated by this formula:

$$SMC\ (\%) = \frac{M_2\text{-}M_3}{M_2\text{-}M_1} \times 100$$

Each result was the average of two replicates and was reported to one decimal place (ISTA, 2018).

3.3.3.2. Pure seed test

According to ISTA (2018), the definition of pure radish seed is intact seeds or a piece of seed larger than one-half the original size including testa attachment. Approximately 50 grams was taken from each sample for the <2.6mm and 2.6-3mm fraction to evaluate pure seed. As the number of seeds >3mm was low, the whole sample was used for the purity test. The working sample was first weighed. The pure seeds were separated from other seeds and inert matter by using a scraper on a purity board. Then each component was weighed and converted to a percentage. The results of each component were adjusted up or down to make sure the total component was 100% (ISTA, 2018). The purity test results were reported as a percentage by weight to one decimal place.

3.3.3.3. Thousand seed weight (TSW) test

TSW test is to determine the weight per 1000 seeds of the sample (ISTA, 2018). TSW was determined for the pure seeds. Eight replicates of 100 randomly selected pure seeds from each sample were weighed. The TSW was calculated from the average weight of the eight replicates and multiplied by 10. As long as the coefficient of variation for the result was below 4.0, the determination is acceptable. The coefficient of variation was determined using the following calculation (ISTA, 2018):

$$\text{Standard deviation } (s) = \sqrt{\frac{N(\sum X^2) - (\sum X)^2}{N \times (N-1)}}$$

X: Weight of each replicate

N: Number of replicates

∑: Sum of

$$\text{Coefficient of variation} = \frac{S}{\overline{X}} \times 100$$

$\overline{X}$: mean weight of 100 seeds

3.3.3.4. Germination test

Germination of pure radish seeds was conducted using the between paper (BP) method (ISTA, 2018). Germination papers were soaked with distilled water for two minutes before testing. Four replicates of 50 randomly selected seeds of each sample were placed between two layers of moist germination papers. Then the bottom of the paper was folded up and the papers were rolled and placed into a zip-seal lock plastic bag to avoid moisture loss. Four germination rolls per sample were placed in a bag and incubated in an incubator (Contherm, Biosym 6000 CP) at a temperature of 20°C for 10 days with 12 hours of light daily. Germination of radish seeds was evaluated by counting the number of normal seedlings (Fig. 3.9A) present at the 4th and 10th day after the beginning of the test. The majority of normal seedlings and badly decayed seedlings were removed at the 4th day. The ungerminated seeds, uncertain, abnormal seedlings (Fig. 3.9B), dead seeds and fresh seeds were evaluated at the 10th day. The germination results were the average of the percentage of normal seedlings of four replicates.

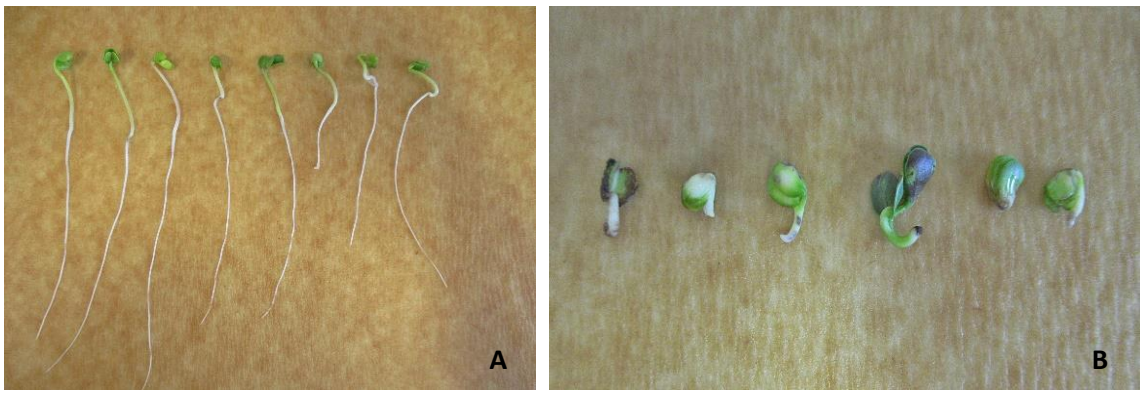


Figure 3.9. Radish seedlings: normal (A) and abnormal (B) on the 10th day. Photos by author.

3.3.3.5. Conductivity test

Seed vigour is used to check the ability of seeds to perform under stress conditions and their potential for storage (ISTA, 2018).

The SMC of each sample was known prior to the conductivity test. Four replicates of 50 randomly selected seeds of each sample were weighed to four decimal places (ISTA, 2018). Radish seeds were placed into plastic containers containing 30ml deionised water. Aluminium foil was then used to cover the containers in order to prevent contamination (Fig. 3.10A). These containers were kept at 20°C ± 2°C for 8 hours (Demir *et al*., 2012). The electrical conductivity of the leachate from imbibed seeds was measured after the 8 hour soaking period by inserting the single electrode cell into the water in the container. The results were recorded whenever "STAR" word on the screen appeared which indicated a stable recording. A single cell electrical conductivity meter (MeterLab, CDM210, Radiometer, Copenhagen) was used in this study (Fig. 3.10B). The conductivity of 30ml a control flask with deionized water kept at the same temperature was also measured. This value was subtracted from the conductivity reading of the steep water from each sample. The results of each sample were the average of four replicates. Therefore for each replicate, conductivity was calculated as below:

$$\text{Conductivity (uS cm}^{-1}\text{ g}^{-1}\text{)} = \frac{\text{Conductivity of sample – conductivity of control water (uS cm}^{-1}\text{)}}{\text{Weight of sample (g)}}$$

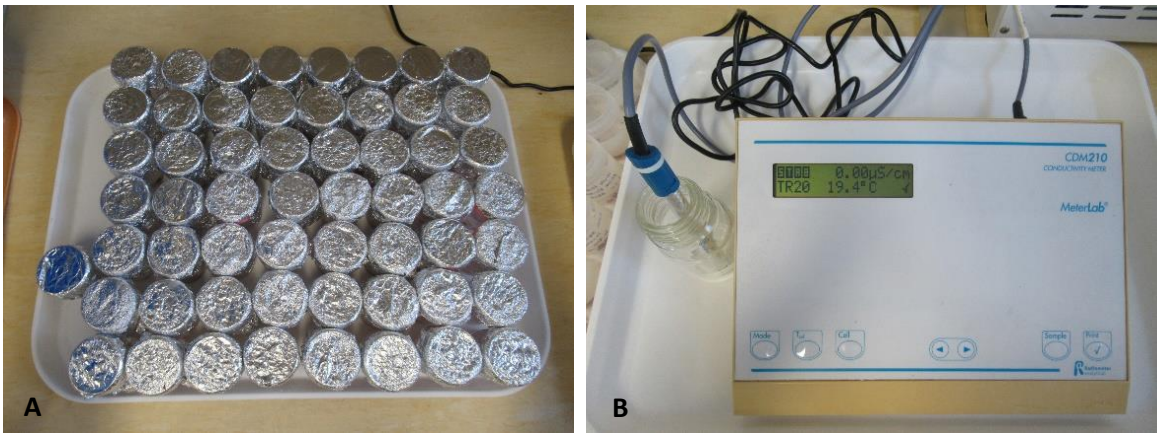


Figure 3.10. Conductivity test. Soaked seeds in the containers (A), conductivity meter (B). Photos by author.

3.3.4 Seed health

Seed health is reported in Chapter 4 and Appendix E.

3.4 Statistical analysis

All data were analysed using an analysis of variance (ANOVA) for RCBD, using Genstat 19th edition (VSN International, Hemel Hempstead, UK). The unrestricted least significant difference (LSD) procedure at 5% significance level was used to test the differences among treatments. The relationship between seed field dressed yield and the percentage of pods damaged by birds was predicted by regression analysis.

3.5 Results

3.5.1 Environmental conditions during the growing season

Environmental conditions were variable during the experiment which ran from 19th September 2018 to 31st March 2019 (Fig. 3.11). Monthly mean relative humidity ranged from 70% to 81%, being lowest during February and highest in October. Total mean rainfall increased steadily from September to February before falling slowly in March. The total rainfall received during the season was 327mm, but monthly mean rainfall varied between 32 mm (in September) and 138 mm (in February). The monthly maximum temperature ranged from 15.2^{o}C in September to 24.3^{o}C in February, the monthly minimum temperature ranged from 5.2^{o}C in September to 13.1^{o}C in January and monthly mean temperature ranged from 10.2^{o}C to 18.5^{o}C in September and January, respectively. In general, temperature and mean rainfall were lowest in September, then increased gradually to reach a peak in January and February. From February to March, temperature and mean rainfall declined. On the other hand, the average relative humidity was higher during the period from September to December and lower from January to February (Fig. 3.11).

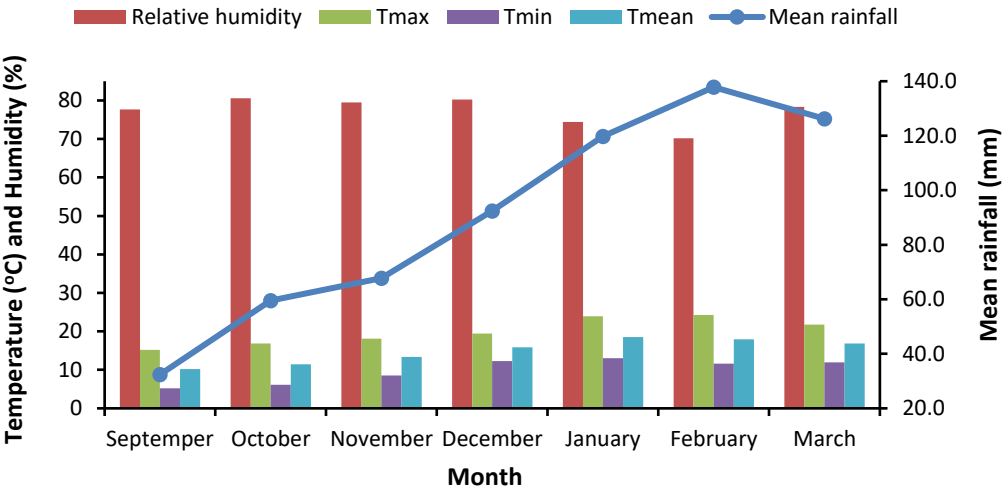


Figure 3.11. Weather data during the growth of the radish seed crop (NIWA, 2019).

3.5.2 Effects of foliar fungicides on white blister disease

At 86 DAS, disease severity was significantly lower than the control for all treatments ($P<0.05$) (Table 3.3). However, by 93 DAS, disease severity for all treatments did not differ from the control (Table 3.3). At 100 DAS, disease severity was significantly lower than the control for treatments 2, 3, 4, 6, 7, 9 and 11, but not for the remaining treatments ($P<0.05$) (Table 3.3).

Treatments 2, 3, 4, 6, 8, 9, 10, 11 and 12 reduced mean staghead lesion length per plant compared with the control at 107 DAS ($P<0.05$) (Table 3.3). It was the same at 114 DAS, except for treatment 4 and 8 ($P<0.05$) (Table 3.3). Treatments 7 and 8 had a significantly larger mean staghead lesion length at 114 DAS ($P<0.05$) (Table 3.3).

Table 3.3. Effects of fungicides on disease severity and mean staghead lesion length.

Treatment	Disease severity (%)			Mean staghead lesion length per plant (mm)	
	86 DAS	93 DAS	100 DAS	107 DAS	114 DAS
1	18.0 [b]	20.5 [a]	34.5 [d]	19.0 [f]	16.8 [c]
2	9.0 [a]	21.5 [a]	24.0 [abc]	5.7 [abc]	5.9 [ab]
3	4.0 [a]	24.5 [a]	20.5 [a]	9.6 [cd]	10.9 [abc]
4	2.0 [a]	26.0 [a]	24.5 [abc]	11.4 [de]	13.1 [bc]
5	6.5 [a]	24.5 [a]	29.5 [bcd]	20.3 [fg]	18.9 [cd]
6	3.0 [a]	28.5 [a]	25.0 [abc]	7.7 [bcd]	8.0 [ab]
7	9.0 [a]	23.5 [a]	21.0 [ab]	16.0 [ef]	25.3 [de]
8	4.5 [a]	26.5 [a]	32.5 [cd]	25.2 [g]	30.3 [e]
9	2.5 [a]	16.5 [a]	21.0 [ab]	4.0 [ab]	5.2 [ab]
10	2.0 [a]	21.5 [a]	26.5 [abcd]	5.4 [abc]	4.9 [a]
11	5.5 [a]	25.0 [a]	25.0 [abc]	2.6 [a]	3.4 [a]
12	3.5 [a]	25.0 [a]	27.0 [abcd]	3.3 [ab]	5.2 [ab]
LSD (5%)	7.8	16.8	8.7	5.0	8.2

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

No treatment reduced the mean percentage of stagheads per plant (Table 3.4), but treatment 8 significantly increased the staghead percentage per plant (P<0.05) (Table 3.4).

The number of infected raceme tips ranged between 1 and 11 (P<0.05), but differences from the control were not significant (Table 3.4).

Ten of the fungicides treatments provided better control of white blister infection on the stem than the control, but treatment 7 did not do so (P<0.05) (Table 3.4).

Mean bulb infection was not reduced by any treatment and treatment 2 actually significantly increased the percentage of infected bulbs (P<0.05) (Table 3.4).

Treatment 8 increased the mean percentage of infected pods compared with the control, but the other treatments did not differ from the control (P<0.05) (Table 3.4).

Table 3.4. Effects of fungicides on infection of inflorescences, raceme tips, stems, bulbs and pods.

Treatment	Mean stagheads per plant (%)	Number of Infected raceme tips	Stems with symptoms (%)	Mean infected bulbs (%)	Mean infected pods (%)
1	23.4 ^{ab}	5.3 ^{ab}	6.1 ^{c}	8.3 ^{a}	4.9 ^{ab}
2	16.8 ^{ab}	6.3 ^{ab}	1.3 ^{a}	33.3 ^{b}	5.3 ^{ab}
3	13.0 ^{ab}	2.3 ^{ab}	1.5 ^{a}	8.3 ^{a}	5.8 ^{ab}
4	10.6 ^{ab}	3.0 ^{ab}	2.2 ^{a}	16.7 ^{ab}	5.3 ^{ab}
5	26.8 ^{b}	5.8 ^{ab}	3.1 ^{ab}	4.2 ^{a}	5.7 ^{ab}
6	23.9 ^{ab}	8.3 ^{ab}	1.6 ^{a}	8.3 ^{a}	5.7 ^{ab}
7	20.0 ^{ab}	10.8 ^{b}	5.2 ^{bc}	8.3 ^{a}	3.8 ^{a}
8	47.6 ^{c}	8.5 ^{ab}	2.3 ^{a}	12.5 ^{a}	8.6 ^{c}
9	9.1 ^{ab}	5.5 ^{ab}	1.2 ^{a}	0.0 ^{a}	4.4 ^{ab}
10	15.4 ^{ab}	8.3 ^{ab}	1.9 ^{a}	12.5 ^{a}	7.0 ^{bc}
11	4.4 ^{a}	1.3 ^{a}	0.8 ^{a}	4.2 ^{a}	5.1 ^{ab}
12	16.4 ^{ab}	8.3 ^{ab}	1.5 ^{a}	12.5 ^{a}	4.2 ^{a}
LSD (5%)	19.8	8.8	2.9	19.4	2.7

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

3.5.3 Effects of foliar fungicides on seed yield

None of the fungicide treatments increased field dressed seed yield or the mean number of seeds per pod compared to the control (P<0.05) (Table 3.5). Field dressed seed yield ranged between 133 g/m^2 and 183 g/m^2. There were about four seeds per pod in this experiment (Table 3.5).

Treatment 5 reduced seed yield per plant compared with the control (P<0.05) (Table 3.5). The average seed yield per plant of all treatments was about 13 g/plant.

Percentage of pods damaged by birds ranged from 6 to 25%. Treatments 2, 3, 9, 10 and 11 had less damage than the control (P<0.05) (Table 3.5).

Table 3.5. Effects of fungicides on seed yield and yield components.

Treatment	Field dressed seed yield (g/m^2)	Seed plant yield (g/plant)	Mean number of seeds per pod	Pod damage by the bird (%)
1	157.5 ^{abcd}	13.4 ^{bc}	4.6 ^{a}	18.2 ^{cd}
2	157.3 ^{abcd}	13.3 ^{bc}	4.2 ^{a}	10.3 ^{ab}
3	163.9 ^{bcd}	14.5 ^{bc}	4.4 ^{a}	10.2 ^{ab}
4	148.9 ^{abc}	12.0 ^{ab}	4.5 ^{a}	11.1 ^{abc}
5	133.4 ^{a}	10.6 ^{a}	3.9 ^{a}	24.5 ^{d}
6	169.8 ^{cd}	14.1 ^{bc}	4.2 ^{a}	14.4 ^{bc}
7	152.5 ^{abc}	12.1 ^{ab}	4.0 ^{a}	16.1 ^{bc}
8	136.0 ^{ab}	12.0 ^{ab}	4.2 ^{a}	13.3 ^{bc}
9	177.0 ^{cd}	13.0 ^{abc}	4.6 ^{a}	5.9 ^{a}
10	165.8 ^{cd}	13.4 ^{bc}	4.0 ^{a}	8.9 ^{ab}
11	172.4 ^{cd}	13.1 ^{abc}	4.5 ^{a}	9.6 ^{ab}
12	182.7 ^{d}	15.0 ^{c}	4.5 ^{a}	11.6 ^{abc}
LSD (5%)	29.0	2.7	0.8	7.3

Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

There was a weak but significant relationship between field dressed seed yield and the percentage of pods damaged by birds (r = 0.64) (Fig. 3.12). The higher the field dressed seed yield, the lower the percentage of pods that had been damaged.

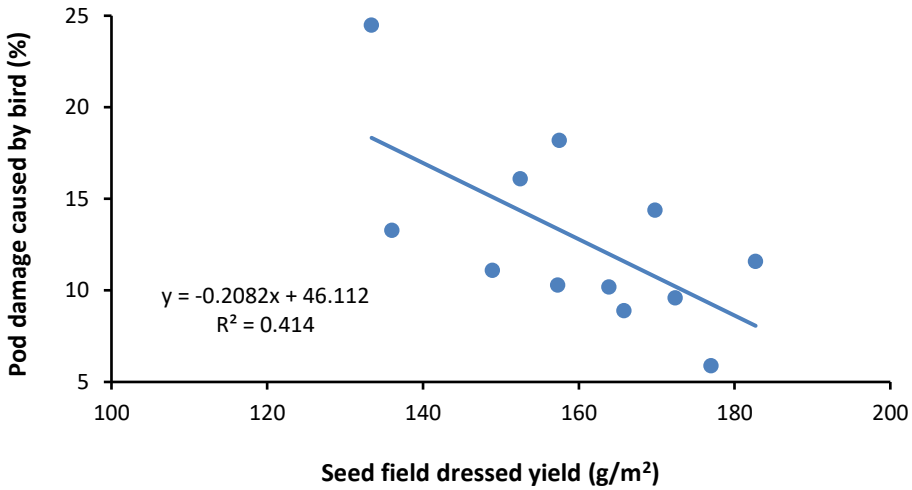


Figure 3.12. Relationship between seed field dressed yield and percentage of pod damage by birds.

Machine dressed seed yield ranged from 100 g/m^2 to 145 g/m^2 (Table 3.6), but none of the treatments outyielded the control. However, treatment 12 had a yield 18% higher than the control, and this was very nearly a significant increase.

Within the seed size categories, for the yield of seeds in the <2.6mm size category (Table 3.6), treatment 5 had a significantly lower weight (8.4 g/m^2) than the control (12.4 g/m^2). For the 2.6-3mm size category, treatment 12 had a significantly greater yield (131.8 g/m^2) than the control (108 g/m^2), but other treatments did not differ significantly from the control ($P<0.05$) (Table 3.6). The yield of seed in the >3mm size category was very low (3 g/m^2 or less), but six treatments significantly out-yielded the control (Table 3.6).

In terms of the interaction contrasts (differences), the difference in machine dressed seed yield between the 2.6-3mm and the >3mm seed size categories was significantly higher ($P<0.05$) for treatment 12 than the control ($P<0.05$) (Table 3.6). Also, the difference in machine dressed seed yield between the 2.6-3mm and the <2.6mm seed size categories was significantly higher for treatments 9 and 12 than the control (Table 3.6). Lastly, the difference in machine dressed seed yield between the <2.6mm and the >3mm seed size categories was significantly lower for treatments 5, 10 and 12 than the control (Table 3.6).

Table 3.6. Effects of fungicides on machine dressed seed yield of different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Machine dressed seed yield (g/m^2)			Total (g/m^2)	Machine dressed seed yield (g/m^2) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)		(2) – (3)	(2) – (1)	(1) – (3)
1	12.4 ^{b}	108.0 ^{abc}	1.4 ^{a}	122.7 ^{abcd}	107.4 ^{abcd}	96.4 ^{ab}	11.0 ^{c}
2	9.4 ^{ab}	110.6 ^{abc}	1.4 ^{a}	121.4 ^{abc}	109.1 ^{bcde}	101.2 ^{bc}	8.0 ^{abc}
3	11.0 ^{ab}	116.4 ^{bcd}	1.6 ^{ab}	128.9 ^{cd}	114.7 ^{cde}	105.4 ^{bcd}	9.3 ^{abc}
4	11.7 ^{b}	113.2 ^{bcd}	2.1 ^{bc}	126.9 ^{bcd}	111.1 ^{cde}	101.4 ^{bc}	9.7 ^{abc}
5	8.4 ^{a}	90.3 ^{a}	1.7 ^{ab}	100.4 ^{a}	88.6 ^{a}	81.9 ^{a}	6.6 ^{a}
6	10.4 ^{ab}	120.4 ^{bcd}	2.4 ^{cd}	133.2 ^{cd}	118.0 ^{cde}	109.9 ^{bcd}	8.0 ^{abc}
7	11.8 ^{b}	106.0 ^{ab}	1.5 ^{ab}	119.3 ^{abc}	104.4 ^{abc}	94.2 ^{ab}	10.2 ^{bc}
8	11.1 ^{ab}	91.9 ^{a}	1.7 ^{ab}	104.7 ^{ab}	90.2 ^{ab}	80.8 ^{a}	9.4 ^{abc}
9	11.0 ^{ab}	127.7 ^{cd}	2.7 ^{de}	141.3 ^{cd}	125.0 ^{de}	116.6 ^{cd}	8.3 ^{abc}
10	10.0 ^{ab}	114.9 ^{bcd}	2.5 ^{cde}	127.4 ^{bcd}	112.4 ^{cde}	104.8 ^{bcd}	7.5 ^{ab}
11	10.7 ^{ab}	120.1 ^{bcd}	2.4 ^{cd}	133.2 ^{cd}	117.7 ^{cde}	109.4 ^{bcd}	8.3 ^{abc}
12	10.5 ^{ab}	131.8 ^{d}	3.0 ^{e}	145.3 ^{d}	128.8 ^{e}	121.3 ^{d}	7.5 ^{ab}
LSD (5%)	3.4	20.7	0.6	23.1	20.5	19.0	3.3

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

Table 3.7. Effects of fungicides on the percentage by weight of seeds in the different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Seed size category (%)			Seed size category (%) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(2) – (3)	(2) – (1)	(1) – (3)
1	10.2 ^{de}	88.6 ^{ab}	1.2 ^{a}	78.4 ^{ab}	87.5 ^{ab}	9.1 ^{d}
2	7.9 ^{ab}	91.0 ^{d}	1.2 ^{ab}	83.1 ^{d}	89.8 ^{c}	6.7 ^{abc}
3	8.5 ^{abcd}	90.3 ^{bcd}	1.3 ^{abc}	81.8 ^{bcd}	89.0 ^{bc}	7.2 ^{bcd}
4	9.2 ^{bcde}	89.2 ^{abcd}	1.7 ^{cde}	80.0 ^{abcd}	87.5 ^{ab}	7.5 ^{bcd}
5	8.3 ^{abcd}	90.0 ^{bcd}	1.7 ^{de}	81.7 ^{bcd}	88.3 ^{abc}	6.6 ^{abc}
6	7.9 ^{ab}	90.3 ^{bcd}	1.9 ^{ef}	82.4 ^{cd}	88.4 ^{bc}	6.0 ^{ab}
7	9.9 ^{cde}	88.9 ^{abc}	1.3 ^{abcd}	79.0 ^{abc}	87.6 ^{ab}	8.6 ^{cd}
8	10.5 ^{e}	87.9 ^{a}	1.6 ^{bcde}	77.4 ^{a}	86.3 ^{a}	8.9 ^{d}
9	7.7 ^{ab}	90.4 ^{bcd}	2.0 ^{ef}	82.7 ^{cd}	88.4 ^{bc}	5.7 ^{ab}
10	7.9 ^{abc}	90.2 ^{bcd}	1.9 ^{ef}	82.3 ^{bcd}	88.3 ^{abc}	6.0 ^{ab}
11	8.0 ^{abc}	90.3 ^{bcd}	1.8 ^{e}	82.3 ^{bcd}	88.5 ^{bc}	6.2 ^{ab}
12	7.1 ^{a}	90.7 ^{cd}	2.2 ^{f}	83.6 ^{d}	88.5 ^{bc}	4.9 ^{a}
LSD (5%)	2.0	2.0	0.4	4.0	2.1	2.1

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

For all the treatments, the majority of the harvested seeds (88-91%) were in the 2.6-3mm size category, with around 11% for <2.6mm and 2% for >3mm size categories (Table 3.7).

Treatments 2 and 12 had a small but significantly higher percentage in the 2.6-3mm size category than the control, while treatments 2, 6, 9, 10, 11 and 12 had a smaller percentage of seeds in the <2.6mm size category than the control (P<0.05) (Table 3.7). All but treatments 2, 3 and 7 had a higher percentage of seeds in the >3mm size category than the control (P<0.05) (Table 3.7).

In terms of the interaction contrasts (differences) for the perentage by weight of seeds in the different seed size categories, the difference between 2.6-3mm and >3mm seed size categories was significantly higher for treatments 2, 6, 9 and 12 than the control ($P<0.05$) (Table 3.7). The difference in the percentage by weight of seeds in the different seed size categories between 2.6-3mm and <2.6mm seed size categories was significantly higher for treatment 2 compared to the control ($P<0.05$) (Table 3.7). On the other hand, the difference in the percentage by weight of seeds in the different seed size categories between <2.6mm and >3mm seed size categories was significantly lower for treatments 2, 5, 6, 9, 10, 11 and 12 than the control ($P<0.05$) (Table 3.7).

3.5.4 Effects of foliar fungicides on seed quality

3.5.4.1. Effects of foliar fungicides on SMC

SMC was obtained from seeds in all three seed size categories. SMC of three seed size categories ranged between 6.0 and 7.7% (Table 3.8). Average SMC of radish seeds was 7.5%, 7.2% and 6.3% in the seed size categories <2.6mm, 2.6-3mm and >3mm, respectively. The fungicide treatments had no effect on SMC in the seed size categories <2.6mm and 2.6-3mm ($P<0.05$) (Table 3.8). Treatments 5 and 6 reduced SMC for >3mm seed size category compared to the control ($P<0.05$) (Table 3.8).

In terms of the interaction contrasts (differences), the difference in the SMC between the <2.6mm and 2.6-3mm seed size categories and between the <2.6mm and >3mm seed size categories was not significantly different between the control and any of the other treatments (Table 3.8). However, the difference in the SMC between the 2.6-3mm and >3mm seed size categories was significantly higher for treatment 6 than the control ($P<0.05$) (Table 3.8).

Table 3.8. Effects of fungicides on SMC in the different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Seed moisture content (%)			Seed moisture content (%) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(1) – (2)	(2) – (3)	(1) – (3)
1	7.5 ^{ab}	7.2 ^{a}	6.4 ^{abc}	0.3 ^{ab}	0.9 ^{a}	1.1 ^{ab}
2	7.4 ^{ab}	7.2 ^{a}	6.2 ^{cd}	0.3 ^{ab}	1.0 ^{ab}	1.2 ^{b}
3	7.5 ^{ab}	7.3 ^{a}	6.4 ^{ab}	0.2 ^{ab}	0.8 ^{a}	1.0 ^{ab}
4	7.6 ^{ab}	7.3 ^{a}	6.3 ^{bcd}	0.3 ^{ab}	1.0 ^{ab}	1.3 ^{b}
5	7.4 ^{ab}	7.1 ^{a}	6.1 ^{de}	0.3 ^{ab}	1.0 ^{ab}	1.3 ^{b}
6	7.4 ^{ab}	7.2 ^{a}	6.0 ^{e}	0.2 ^{ab}	1.2 ^{b}	1.3 ^{b}
7	7.2 ^{b}	7.2 ^{a}	6.4 ^{ab}	0.0 ^{a}	0.8 ^{a}	0.8 ^{a}
8	7.4 ^{ab}	7.1 ^{a}	6.2 ^{cd}	0.3 ^{ab}	0.9 ^{a}	1.2 ^{ab}
9	7.5 ^{ab}	7.2 ^{a}	6.3 ^{abc}	0.3 ^{ab}	0.9 ^{a}	1.2 ^{ab}
10	7.7 ^{a}	7.3 ^{a}	6.4 ^{abc}	0.4 ^{b}	0.9 ^{ab}	1.3 ^{b}
11	7.8 ^{a}	7.4 ^{a}	6.5 ^{a}	0.4 ^{b}	0.9 ^{a}	1.3 ^{b}
12	7.7 ^{a}	7.3 ^{a}	6.5 ^{ab}	0.4 ^{b}	0.9 ^{a}	1.3 ^{b}
LSD (5%)	0.5	0.3	0.2	0.4	0.3	0.5

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

3.5.4.2. Effects of foliar fungicides on percentage pure seed

The purity of seed in the three seed size categories ranged from 71% to 93% (Table 3.9). Purity in the seed size categories <2.6mm and >3mm was lower by 10-20% than in the seed size category 2.6-3mm (Table 3.9).

Seven of the fungicide treatments had a significantly lower purity than the control in the <2.6mm seed size category and treatment 11 had a significantly lower purity than the control

in the 2.6-3mm size category (Table 3.9). However, there were no differences in purity within the >3mm seed size category (Table 3.9).

Table 3.9. Effects of fungicides on the percentage pure seed of different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Pure seed (%)			Pure seed (%) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(2) – (1)	(2) – (3)	(1) – (3)
1	81.8 ^{a}	92.9 ^{a}	75.6 ^{ab}	11.1 ^{a}	17.4 ^{abc}	6.3 ^{bc}
2	77.0 ^{ab}	91.2 ^{ab}	75.0 ^{ab}	14.3 ^{ab}	16.3 ^{abc}	2.0 ^{abc}
3	75.5 ^{ab}	91.8 ^{ab}	83.1 ^{a}	16.3 ^{ab}	8.7 ^{a}	-7.6 ^{ab}
4	72.3 ^{b}	92.0 ^{ab}	81.8 ^{a}	19.7 ^{b}	10.2 ^{ab}	-9.5 ^{a}
5	71.5 ^{b}	92.0 ^{ab}	77.9 ^{ab}	20.5 ^{b}	14.1 ^{abc}	-6.3 ^{abc}
6	70.6 ^{b}	90.2 ^{ab}	76.4 ^{ab}	19.6 ^{b}	13.7 ^{abc}	-5.8 ^{abc}
7	76.1 ^{ab}	91.7 ^{ab}	69.4 ^{b}	15.5 ^{ab}	22.3 ^{bc}	6.7 ^{bc}
8	74.7 ^{b}	90.1 ^{ab}	67.1 ^{b}	15.8 ^{ab}	23.4 ^{c}	7.6 ^{c}
9	73.7 ^{b}	92.0 ^{ab}	76.8 ^{ab}	18.3 ^{b}	15.2 ^{abc}	-3.1 ^{abc}
10	75.8 ^{ab}	91.9 ^{ab}	78.8 ^{ab}	16.1 ^{ab}	13.1 ^{abc}	-3.1 ^{abc}
11	72.4 ^{b}	89.1 ^{b}	82.1 ^{a}	16.7 ^{ab}	7.1 ^{a}	-9.7 ^{a}
12	72.4 ^{b}	90.8 ^{ab}	84.1 ^{a}	18.4 ^{b}	6.7 ^{a}	-11.7 ^{a}
LSD (5%)	6.9	3.2	12.3	6.9	12.9	14.6

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letters in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

In terms of the interaction contrasts (differences), the difference in the purity of seeds between the 2.6-3mm and <2.6mm seed size categories was significantly higher for treatments 4, 5, 6, 9 and 12 than the control (P<0.05) (Table 3.9). Moreover, the difference in the purity of seeds between <2.6mm and >3mm seed size categories was significantly different between treatments 4, 11 and 12 and the control (P<0.05) (Table 3.9). On the other hand, the difference in the purity of seeds between the 2.6-3mm and >3mm seed size

categories was not significantly different between the control and any of the other treatments (Table 3.9).

3.5.4.3. Effects of foliar fungicides on TSW

Table 3.10. Effects of fungicides on thousand seed weight in different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Thousand seed weight (g)			Thousand seed weight (g) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(2) – (1)	(3) – (2)	(3) – (1)
1	7.0 ^{a}	11.8 ^{abcd}	13.7 ^{ab}	4.8 ^{ab}	1.9 ^{ab}	6.7 ^{ab}
2	6.9 ^{a}	11.8 ^{abcd}	14.0 ^{a}	4.9 ^{ab}	2.3 ^{b}	7.1 ^{ab}
3	7.0 ^{a}	11.9 ^{abc}	14.0 ^{a}	4.9 ^{ab}	2.1 ^{b}	7.0 ^{ab}
4	6.5 ^{a}	11.7 ^{bcd}	13.9 ^{a}	5.2 ^{b}	2.2 ^{b}	7.4 ^{b}
5	6.7 ^{a}	11.7 ^{bcd}	14.0 ^{a}	5.0 ^{ab}	2.3 ^{b}	7.3 ^{b}
6	7.0 ^{a}	11.7 ^{bcd}	13.9 ^{a}	4.8 ^{ab}	2.2 ^{b}	7.0 ^{ab}
7	6.9 ^{a}	11.4 ^{d}	13.3 ^{b}	4.5 ^{a}	1.9 ^{ab}	6.4 ^{a}
8	6.6 ^{a}	11.5 ^{cd}	13.6 ^{ab}	4.9 ^{ab}	2.1 ^{b}	7.0 ^{ab}
9	6.9 ^{a}	11.8 ^{abcd}	13.9 ^{a}	4.9 ^{ab}	2.1 ^{b}	7.0 ^{ab}
10	6.8 ^{a}	12.1 ^{a}	13.7 ^{ab}	5.4 ^{b}	1.6 ^{a}	7.0 ^{ab}
11	6.6 ^{a}	11.8 ^{abc}	14.0 ^{a}	5.3 ^{b}	2.1 ^{b}	7.4 ^{b}
12	7.0 ^{a}	12.2 ^{ab}	14.1 ^{a}	5.3 ^{b}	2.0 ^{b}	7.4 ^{b}
LSD (5%)	0.5	0.4	0.5	0.7	0.4	0.8

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

The average TSW was 6.8g, 11.8g and 13.8g for seeds in categories <2.6mm, 2.6-3mm and >3mm, respectively (Table 3.10). TSW for all treatments did not differ significantly from the control for any of the three seed size categories (Table 3.10).

In terms of the interaction contrasts (differences), the difference in the TSW between any two of the seed size categories was not significantly different between the control and any of the other treatments (Table 3.10).

3.5.4.4. Effects of foliar fungicides on percentage germination

Table 3.11. Effects of fungicides on germination of seeds in different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Germination (%)			Germination (%) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(1) – (2)	(3) – (2)	(3) – (1)
1	94.0 [a]	94.5 [ab]	94.0 [a]	-0.5 [a]	-0.5 [a]	0.0 [ab]
2	94.5 [a]	94.5 [ab]	88.8 [a]	0.0 [a]	-5.8 [a]	-5.8 [a]
3	94.5 [a]	96.0 [ab]	93.3 [a]	-1.5 [a]	-2.8 [a]	-1.3 [ab]
4	90.0 [a]	88.8 [a]	89.8 [a]	1.3 [a]	1.0 [a]	-0.3 [ab]
5	92.0 [a]	93.5 [ab]	89.0 [a]	-1.5 [a]	-4.5 [a]	-3.0 [ab]
6	93.0 [a]	91.5 [ab]	92.3 [a]	1.5 [a]	0.8 [a]	-0.8 [ab]
7	95.5 [a]	98.0 [b]	94.5 [a]	-2.5 [a]	-3.5 [a]	-1.0 [ab]
8	94.0 [a]	94.0 [ab]	88.5 [a]	0.0 [a]	-5.5 [a]	-5.5 [a]
9	91.0 [a]	92.0 [ab]	93.8 [a]	-1.0 [a]	1.8 [a]	2.8 [b]
10	93.0 [a]	96.0 [ab]	91.3 [a]	-3.0 [a]	-4.8 [a]	-1.8 [ab]
11	89.5 [a]	92.0 [ab]	89.8 [a]	-2.5 [a]	-2.3 [a]	0.3 [ab]
12	95.0 [a]	93.0 [ab]	94.0 [a]	2.0 [a]	1.0 [a]	-1.0 [ab]
LSD (5%)	7.5	7.5	7.8	6.6	8.5	6.3

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

The percentage germination of radish seeds ranged from 89% to 98% (Table 3.11). The percentage germination in the seed size categories <2.6mm and 2.6-3mm were higher than the germination in the seed size category >3mm. There were no significant differences in

germination between the control and any of the treatments within any of the seed size categories (Table 3.11).

In terms of the interaction contrasts (differences), the difference in the TSW between any two of the seed size categories was not significantly different between the control and any of the other treatments (Table 3.11).

3.5.4.5. Effects of foliar fungicides on conductivity

Seed vigour of radish seeds was assessed by the conductivity test. Seeds in size category 2.6-3mm had a lower conductivity than seeds in size categories <2.6mm and >3mm (Table 3.12). Treatment 7 increased conductivity in the seed size category <2.6mm and treatment 11 decreased it in the seed size category 2.6-3mm compared to the control ($P<0.05$) (Table 3.12). Treatments 3, 4, 5, 9 and 12 decreased conductivity by 24 to 37 $uScm^{-1}g^{-1}$ in the seed size category >3mm compared to the control ($P<0.05$) (Table 3.12).

In terms of the interaction contrasts (differences), the difference in the conductivity between the <2.6mm and 2.6-3mm seed size categories was significantly higher for treatments 5, 7 and 11 than control ($P<0.05$) (Table 3.12). Furthermore, the difference in the conductivity between the >3mm and 2.6-3mm seed size categories was significantly lower for treatment 3 than the control ($P<0.05$) (Table 3.12). The difference in the conductivity between the <2.6mm and >3mm seed size categories was significantly higher for treatments 2, 3, 4, 5, 10 and 12 than the control ($P<0.05$) (Table 3.12).

Table 3.12. Effects of fungicides on the conductivity of seeds in different seed size categories and their interactions with seed size category assessed by testing whether each difference between seed size categories, differs between treatments.

Treatment	Conductivity seed (uS cm-1 g-1)			Conductivity seed (uS cm-1 g-1) differences[1]		
	<2.6mm (1)	2.6 – 3mm (2)	> 3mm (3)	(1) – (2)	(3) – (2)	(1) – (3)
1	139.1 bcd	130.2 a	154.8 ab	8.9 a	24.6 b	-15.7 a
2	143.9 abcd	155.0 ab	132.0 bc	28.9 ab	17.0 ab	11.9 b
3	128.8 d	121.5 ab	117.8 c	7.3 a	-3.7 a	11.0 b
4	138.0 bcd	112.8 ab	121.8 c	25.2 ab	9.0 ab	16.2 b
5	153.0 abc	112.0 ab	131.2 c	41.0 b	19.2 ab	21.8 b
6	134.9 cd	108.4 ab	134.4 abc	26.5 ab	26.0 b	0.5 ab
7	162.8 a	124.2 ab	156.6 a	38.5 b	32.3 b	6.2 ab
8	146.7 abcd	116.3 ab	139.0 abc	30.4 ab	22.7 ab	7.7 ab
9	132.8 d	114.8 ab	129.2 c	18.0 ab	14.4 ab	3.6 ab
10	155.5 ab	125.9 ab	137.2 abc	29.7 ab	11.4 ab	18.3 b
11	145.8 abcd	104.7 b	139.0 abc	41.0 b	34.3 b	6.8 ab
12	141.7 bcd	113.5 ab	124.3 c	28.3 ab	10.8 ab	17.5 b
LSD (5%)	20.2	26.6	23.5	23.4	27.8	24.1

[1] Each of the last three columns tests one component of the interaction between treatment and seed size category. Within a column, treatments with a letter in common do not differ significantly (P<0.05).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

3.6 Discussion

3.6.1 White blister disease

Temperatures during crop growth (Fig. 3.11) were those that favour oospore and zoospore germination (Liu, 1992; Gilijam *et al*., 1998; Verma & Bhowmik, 1989) and relative humidity higher than 75% (Fig. 3.11) favours *A. candida* development (Saharan, 1984; Kolte, 1985; Lakra & Saharan, 1988; Verma & Bhowmik, 1989). Rainfall in January and February (Fig. 3.11) and

overhead irrigation is also highly likely to have aided zoospore movement throughout the crop.

Disease severity was initially significantly lower than the control for Ridomil Gold MZ WG, Cobra and the fungicide mixtures involving Foschek plus Metylaxyl (treatment 7), Ranman plus Mancozeb plus Amistar followed by Ranman plus Mancozeb plus Seguris Flexi, then Ranman plus Mancozeb, then Ridomil Gold plus Pristine and finally Ridomil Gold (treatment 9), Ranman plus Mancozeb followed by Ranman plus Mancozeb plus Seguris Flexi then Ranman plus Mancozeb plus Amistar, then Ridomil Gold MZ WG plus Pristine and finally Ridomil Gold MZ WG (treatment 11) at the later assessments. However, none of the treatments reduced the percentage of stagheads per plant, the number of infected raceme tips, the percentage of infected bulbs or the number of infected pods, but the percentage of stems with symptoms was significantly reduced by all the fungicide treatments except for Metalaxyl-M and Foschek plus Metalaxyl-M.

Metalaxyl-M plus mancozeb has been used overseas for control of white blister disease (Verma & Petrie, 1979; Mathur & Bhatnagar, 1991; Minchinton *et al*., 2004; Patnude & Nelson, 2013) and is regarded as the industry standard for white blister and other oomycete pathogens (CABI, 2016). In New Zealand, this treatment (Ridomil Gold MZ WG) did not reduce the percentage of infected radish racemes in a field trial in the 2015-2016 season but did so in a field trial in the 2016-2017 season (Braithwaite *et al*., 2018). This inconsistent response was reflected in the present trial where Ridomil Gold MZ WG (treatment 2 and 3) reduced disease severity but not stagheads or infected racemes.

Earlier work (FAR, 2016) in the 2013-2014 season found that Max CL (treatment 6) reduced the incidence of white blister disease, but the response did not occur in the 2015-2016 season field trial of Braithwaite *et al.* (2018) or in the present trial.

Of all the treatments, the multi-fungicide applications (treatments 9, 10, 11 and 12) produced the best results for reducing white blister attack in mean staghead lesion length per plant, and treatments 7, 9 and 11 also significantly reduced the disease severity. This approach was also used by in the 2016-2017 season where Amistar followed by Ridomil Gold MZ WG plus Amistar reduced the percentage of diseased plants, as did Pristine, Ranman and Seguris Flexi plus Amistar. Amistar (Azoxystrobin) was also recommended for control of *A. candida* in Hawai'I

(Patnude & Nelson, 2013). Similarly, mixing of Amistar and Ridomil has been used in horseradish and Indian mustard crops (Minchinton *et al.*, 2004; Gairola & Tewari, 2019).

Foschek did reduce disease in the 2016-2017 season but also reduced seed yield (FAR, 2017). On the other hand, the combination of Foschek and Metalaxyl-M reduced disease severity in the 2018-2019 season by up to 13.5%. It meant Foschek could be a potential fungicide to control *A. candida* if Foschek is combined with Metalaxyl-M.

3.6.2 Seed yield

Braithwaite *et al*. (2018) reported significant radish seed yield increases following fungicide application in both the 2015-2016 and 2016-2017 growing season. In the first season Ridomil Gold MZ WG, Amistar and Pristine increased seed yield by between 15% to 24% over that of the untreated control, with Amistar producing the greatest yield (788 kg/ha). In the following season, Ridomil Gold MZ WG, Pristine, Seguris Flexi plus Amistar and Amistar plus Ridomil Gold MZ WG also significantly increased seed yield by between 35% to 66%, with the Seguris Flexi plus Amistar treatment producing the greatest yield (1558 kg/ha). In the 2018-2019 season, none of the fungicide treatments increased seed yield, either field dressed or machine dressed, although the multi-fungicide regime of treatment 12 only just missed being significant ($P<0.05$) for machine dressed seed yield with an 18% increase. However, after seed size grading, this treatment outyielded the control in the 2.6-3mm seed size category (by 22%); 91% of the machine dressed seeds yield were in this category. In a commercial seed lot, seeds in the <2.6mm size category would be discarded (Khan, 2019). As there were no differences in the number of seeds per pod, this yield increase must have been the result of the small increase in thousand seed weight (TSW for treatment 12 was higher than for the control, but not significantly so).

3.6.3 Seed quality

Quality of seeds in the 2.6-3mm seed size category did not differ among from the treatments for purity, germination or TSW. Treatment 11 had a higher vigour than the control as assessed by the conductivity test. There were some variations among quality parameters within the >3.0mm seed size category, but as this group constituted 3% or less of the total, those differences are of no practical importance.

3.7 Conclusion

While Metalaxyl-M plus Mancozeb (Ridomil Gold MZ WG) is widely used for control of white blister overseas, resistance to this fungicide by other oomycete pathogens has been reported (Cook & Zhang, 1985; Molinero-Ruiz *et al*., 2003; Matson *et al*., 2015). Although not yet reported for white blister, there is a concern that this could develop (Braithwaite *et al*., 2018). Braithwaite *et al*. (2018) suggested that the use of fungicides mixes rather than Ridomil Gold MZ WG alone would offer industry a good anti-resistance strategy, by alternating or combining chemicals with different modes of action. In the current trial, this alternative approach was not successful in terms of reducing staghead production, infected racemes or increasing seed yield. A factor not discussed by Braithwaite *et al*. (2018) was the cost of this multi-fungicide approach to white blister control. At least for the 2018-2019 season, it would have been an expensive exercise with no financial return to the grower as compared to not applying fungicides.

Chapter 4

Oospore contamination of radish seed and determining the presence of *Albugo candida* in the plant

4.1 Introduction

Albugo candida is an obligate biotroph pathogen (Lamour & Kamoun, 2009) which infects a large number of plant species including vegetable crops and weeds, especially in the Capparaceae, Cleomaceae, Aizoaceae and Brassicaceae families (Saharan & Verma, 1992; Choi *et al*., 2009; McMullan *et al*., 2015). These include *B. juncea*, *B. oleracea*, *R. sativus*, *Capparis spinose*, *Aubrieta deltoidea*, *Alyssum saxatile*, *Lunaria annua*, and *Cleome hassleriana* as well as wild species such as *A. thaliana*, *C. bursa-pastoris*, *Sisymbrium officinale* and *Cleome anomala* (Saharan & Verma, 1992; Jouet, 2016). In Australia, Minchinton *et al*. (2004) reported the presence of *A. candida* on *C. bursa-pastoris*, *B. rapa*, *R. sativus* and *B. oleracea*. In Holland, *A. candida* was reported on cabbage and Brussels sprout (Gilijamse *et al*., 1998). The pathogen can infect all the parts of the plant, causing yield losses. In India, yield losses from 17 to 60% for *B. juncea* and *B. rapa* were reported by Awasthi *et al*. (2012) and Bisht *et al*. (2016).

In New Zealand, *A. candida* has been reported on *B. oleracea* (Baker, 1955; Hill, 1979; Pennycook, 1989), *B. campestris, B. rapa, C. bursa-pastoris, Cardamine* sp., *Cleome spinosa, Lepidium oleraceum, L. ruderale, L. sativum, Malcomia maritima, Rorippa islandica* and *Sisymbrium officinale* (Baker, 1955) and *R. sativus* (Baker, 1955; Boesewink *et al*., 1977). Dingley (1969) reported that the disease was common throughout New Zealand but only of minor economic importance. However, the disease has now spread to most radish seed production fields in New Zealand (McKay, pers. com., 2019), causing losses in seed yield from 20% to 70% (Taylor, pers. com., 2019). At harvest, if the crop is grown for seed production, oospores are freed from the staghead tissues and contaminate seeds. Also, plant debris containing mature oospores is ploughed in and contaminates the soil. The pathogen can persist in lateral roots, debris and in the soil for at least 20 years under dry conditions (Kadow & Anderson, 1940; Endo & Linn, 1960; Verma & Petrie, 1975; Tewari & Skoropad, 1977; Agrios, 2015).

Molecular methods have now been applied to the study of the genetic variability of various pathogen populations because compared with traditional methods, they are much faster, more specific, sensitive, and accurate, and can be performed and interpreted by personnel with no specialized taxonomical expertise. Additionally, these techniques allow the detection and identification of non-culturable microorganisms, and due to their high degree of specificity, molecular techniques can distinguish closely related organisms at different taxonomic levels (Capote *et al*., 2012).

Albugo candida is a seed-borne pathogen (Petrie, 1975; Verma & Petrie, 1980; Jacobson *et al*., 1998; Minchinton, 2007) but whether the oospores are only carried on the seed externally, or the pathogen can survive within the plant to infect the developing seed internally is yet to be conclusively demonstrated. The objectives of this chapter were to evaluate (i) the location of oospores on or in the seeds (either internal and/or external), (ii) that radish, forage brassica (turnip) and the weed *C. bursa-pastoris* (shepherd's purse) plant samples showing symptoms of white blister were actually infected by *A. candida*; (iii) the internal presence of *A. candida* in plant parts including developing seeds. A pair of primers was used to amplify the protein kinase G gene region which is present in *A*. *candida* but not in the plants. It was hypothesised that *A. candida* is present endophytically in radish.

4.2 Materials and methods

4.2.1 Isolation and quantification of oospores from seeds

Commercially graded radish seeds (2.6-3mm size category) were used to assess the presence of *A. candida* oospores using a washing and filtration method (Petrie,1975; Minchinton *et al*., 2004; FAR, 2017). Five-gram seed subsamples were taken at random from each of the four replicates of the 12 treatments (48 subsamples in total) from the field trial (see Chapter 3). First, 10ml sterile distilled water mixed with 200µL Tween 20 were poured into a flask containing 50 seeds and then vortexed for 2 minutes at 2000 rpm (Labnet, VX-200 Vortex Mixer). Seed samples were then separated from the washing liquid by sieving before adding 10ml of sterile deionised water to rinse the seeds. Then, the washing liquid was poured carefully into the filtering apparatus equipment. The equipment included a magnetic filter funnel (47mm diameter, 500ml capacity) used with a filter flask attached to a side arm of 1000ml. A vacuum pump (Welch, model 2534C-02) was connected to the container (Fig 4.1) and oospores were collected by suction on the filter discs (47mm diameter, 0.45µm spore

size). Filter discs were air-dried for 15 minutes and cleared with 3-4 drops mineral oil (Fig. 4.2). Finally, the oospores were observed and counted the whole cleared filter discs under a light microscope (1000X magnification) guided by Mark Braithwaite at Plant Diagnostics Ltd., Templeton (Fig. 4.3) (Petrie, 1975; Minchinton *et al*., 2004).

The mean number of oospores per gram of seed were calculated by dividing the mean number of oospores by five. To express the results as the mean number of oospores per seed, the mean number of oospores per gram of seed were divided by the number of seed per gram. The number of seeds per gram was calculated using the thousand seed weight in Table 3.10.

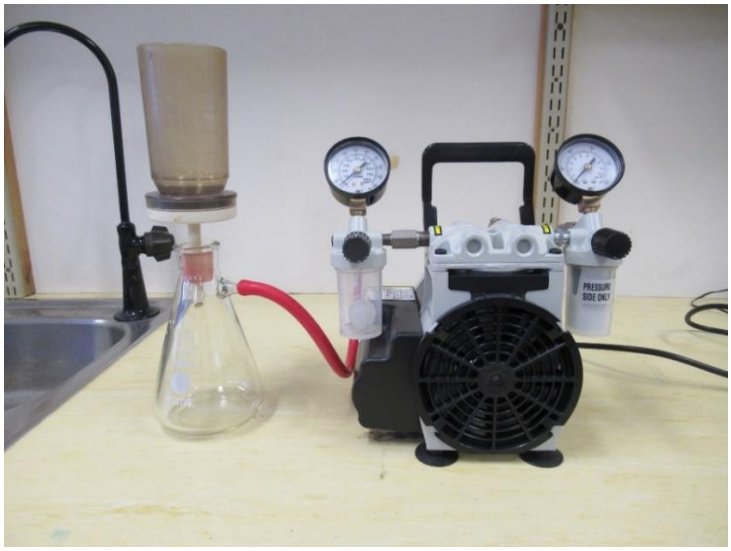


Figure 4.1. Filtering apparatus equipment. Photo by author.

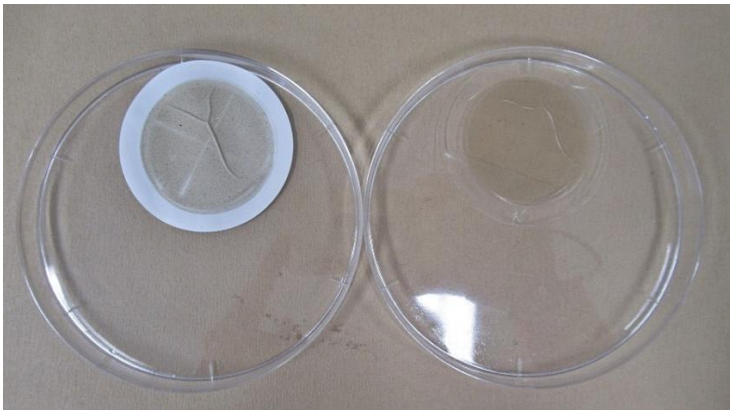


Figure 4.2. Filter disc before (left) and after (right) being cleared by mineral oil. Photo by author.

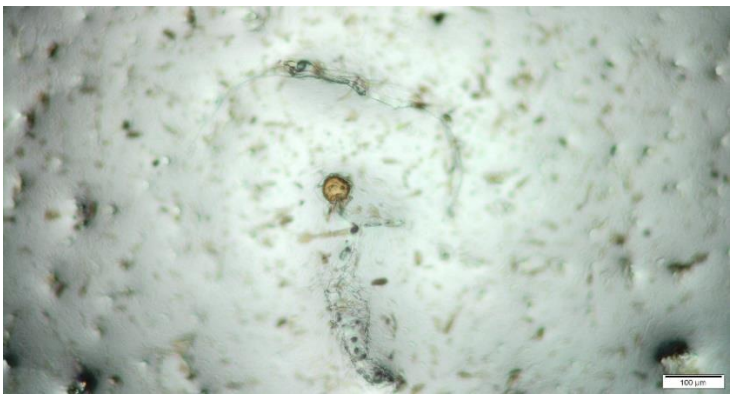


Figure 4.3. Image of an oospore of *A. candida*, 1000X magnification. Photo by author.

4.2.2 Molecular identification of *A. candida* in Brassica species

Samples of infected plant tissues and seeds (Table 4.1) were collected from the radish field trial (see Chapter 3) and glasshouse trials (see Chapter 2), an infected turnip seed crop and an infected shepherd's purse plant (Fig. 4.4, 4.5).

Table 4.1. Samples collection.

Number of samples	Place of collection	Host plant species	Plant parts assessed
68	Field trial	Radish	Leaf, pod, bulb, root, seed, stem, staghead.
55	Glasshouse trials	Radish	Leaf, pod, stem.
3	Seed production field	Turnip	Stem.
1	Field	Shepherd's purse	Stem.

Figure 4.4. Infected shepherd's purse plant sample. Photo by author.

Figure 4.5. Infected samples from the field trial and glasshouse trials: leaf (A), stem (B), stagheads (C), bulbs and roots (D), pods (E), developing pods (F) and seeds (G). Red arrows indicated infected samples. Photos by author.

4.2.2.1. DNA extraction

The DNA extraction was carried out using two methods: Chelex® resin and a Genomic DNA Mini Kit (Plant) (GeneAid, Taiwan) to compare if DNA yielded from plant tissues using Chelex® is the same as the Kit method.

The Chelex® method was used to extract DNA from sori (spore masses), infected plants, lesioned plant tissues and seeds.

- Inoculation loops were used to collect sori from the infected leaves, stems, pods, and stagheads (Fig. 4.4, 4.5A, B, C, E, F). Around 3 mm diameter of sori were collected from each tissue and transferred to a 1.5ml microcentrifuge tube. 500µl Chelex® resin (5% w/v) was then added and the content was vortexed for 5 seconds at 2000 rpm (Labnet, VX-200 Vortex Mixer) (Sepp *et al*., 1994) before adding 1 ml liquid nitrogen to each tube. The samples were then ground manually using a micro pestle and were placed in boiling water for 10 minutes. After this, each tube was centrifuged for 4 minutes at 16,000 x g and each supernatant was carefully transferred to a new 1.5ml microcentrifuge tube which was then stored at -20°C as the sori DNA template (Möhlenhoff *et al*., 2001).

- To extract DNA from plant lesions and seeds (Fig. 4.4, 4.5), 500mg of each lesioned tissues including leaves, pods, stems, stagheads, bulbs, roots and seeds were cut by a scalpel. Each sample then was ground to a fine powder with a mortar and pestle after adding 4 ml of liquid nitrogen (Jacobson *et al*., 1998; Allen *et al*., 2006). Then 1000µl Chelex® resin (5% w/v) was added to each sample and thoroughly mixed. The content was transferred to a 1.7ml microcentrifuge tube, heated in boiling water for 10 minutes and other stages were carried out as described above (Jacobson *et al*., 1998; Hennequin *et al*., 1999; Möhlenhoff *et al*., 2001; Walsh *et al*., 2013; Alizadeh *et al*., 2017).

The Genomic DNA Mini Kit method was also used to extract genomic DNA from *A. candida* in the lesioned plant tissues (Fig. 4.4, 4.5) and seeds according to the manufacturer's protocol. Around 100mg of frozen lesioned plant tissue or seeds or 25mg of lesioned dry sample was exposed to liquid nitrogen and ground to a fine powder. Each sample was transferred to a 1.5ml microcentrifuge tube and 400µL of GP1 Buffer and 5µL of RNase A were added into the sample tube and mixed thoroughly by vortex before being incubated in a thermos shaker for 10 minutes at 60°C. During incubation, the sample tube was inverted every 5 minutes. 100µL

of GP2 Buffer was added to the tube mixed thoroughly by vortex, then incubated on ice for 3 minutes. After incubating, the entire contents of the microcentrifuge tube were transferred to a filter column placed in a 2ml collection tube, centrifuged at 1,000 x g for 1 min then the filter column was discarded. The flow-through from the 2ml collection tube was transferred to a new 1.5ml microcentrifuge tube and 1.5 volume of GP3 Buffer was added. The sample was mixed vigorously by vortex for 5 seconds. The GD column was placed in a new 2ml collection tube, 700µL of the sample was transferred to the GD column, centrifuged at 15,000 x g for 2 minutes, then the flow-through was discarded. This stage was repeated until all remaining mixture had been transferred to the GD column. The GD column was transferred to a new 2ml collection tube and 400µL of W1 Buffer was added to the column, centrifuged at 15,000 x g for 30 seconds. The flow-through was then discarded and the GD column replaced in the 2ml collection tube. In the next stage, 600 µL of Wash Buffer was added to the GD column and centrifuged at 15,000 x g for 30 seconds, then the flow-through was discarded and the GD column replaced in the 2ml collection tube. After discarding the filtrate, the GD column was again centrifuged at 15,000 x g for 3 min. The DNA was eluted into a fresh 1.5mL microcentrifuge tube by adding 100µL of pre-heated Elution Buffer (depending on the weight of sample at the beginning) to the dried GD column for 5 min at room temperature and then centrifuging at 15,000 × g for 30 seconds.

All seeds samples were checked for the presence of *A. candida* internally after two washing methods:

i) Seeds after washing as described in 2.2.1 to count the number of oospores were used to extract DNA. Three grams of non-symptomatic seed and three grams of shrivelled seed were used.

ii) Three grams of randomly selected seeds of each treatment were soaked in 5% sodium hypochlorite for two minutes, followed by five washes in sterile distilled water.

DNA sample concentrations of all the extracts were determined using a Nanodrop spectrophotometer (Thermo Scientific, Germany), using 1µL of each sample. DNA quantification was expressed as ng/µl (Hennequin *et al*., 1999; Walsh *et al*., 2013; Alizadeh *et al*., 2017). The purified DNA then was stored at -20°C.

4.2.2.2. PCR amplification and sequencing

To identify *A. candida*, the protein kinase G gene region was amplified using a standard polymerase chain reaction protocol. The amplification primers were PKG-F (5'-GCCGTCGACGTGATATCTTTGC-3') and PKG-R (5'-GCGATCACATCGGCTTGTCGTGG-3') (Jouet, 2016). The internal transcribed spacer ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') region of the ribosomal operon were first used (Riit *et al*., 2016; Alizadeh *et al*., 2017) but this also amplified the gene region of the plant. Protein kinase G gene region does not exist in plants (Uhler, 1993; Sokolowski *et al*., 2017; Kelly *et al*., 2018). Each PCR reaction contained a final volume of 25µL, comprised of a number of components (Table 4.2). PCR thermal cycling conditions for protein linase G were as follows: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturing at 95°C for 40 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes (Jouet, 2016; Alizadeh *et al*., 2017).

Table 4.2. Chemical component for each PCR reaction mixture sample (Alizadeh *et al*., 2017).

No.	Chemical component	Amount per sample (µL)
1	PCR buffer (10×)	2.50
2	dNTP mix (2.5 mM)	2.00
3	F- primer (10 µM)	1.00
4	R- primer (10 µM)	1.00
5	Ultrapure distilled water	16.25
6	Taq DNA polymerase	0.25
7	Template DNA	2 .00

To check the presence of PCR products, five microliter aliquots of each amplification PCR product were loaded on 1% agarose gels and electrophoresed at a constant voltage of 120V for 50 minutes. The DNA bands then were visualised and photographed under UV light using an UVItec gel documentation system (model UVIdoc HD2; UVItec Cambridge, UK). The samples along with primers were then sequenced at the Bio-Protection Research Centre, Lincoln University. Sequence files generated from PKG sequencing were edited and assembled using the Chromas Pro software and BLAST (basic local alignment search tool) analysis for nearest relatives was performed through https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome US National Centre for Biotechnology Information (NCBI) to find the nearest relatives (Alizadeh *et al*., 2017).

4.2.3 *Albugo candida* internal transmission in radish plants

The Genomic DNA Mini Kit method was used to evaluate internal transmission of *A. candida* in radish plants. Twenty samples from each healthy stem (Fig. 4.6 blue arrows) without any symptom of the white blister disease, twenty segments from abnormal plant parts (Fig. 4.6 red arrows) and five segments from galls on the stem (Fig. 4.6 yellow arrow) were randomly selected and assessed. The pathogen was considered responsible for abnormality on plant parts which appeared on stems and inflorescences as well as galls on the stems (Fig. 4.6 red and yellow arrows).

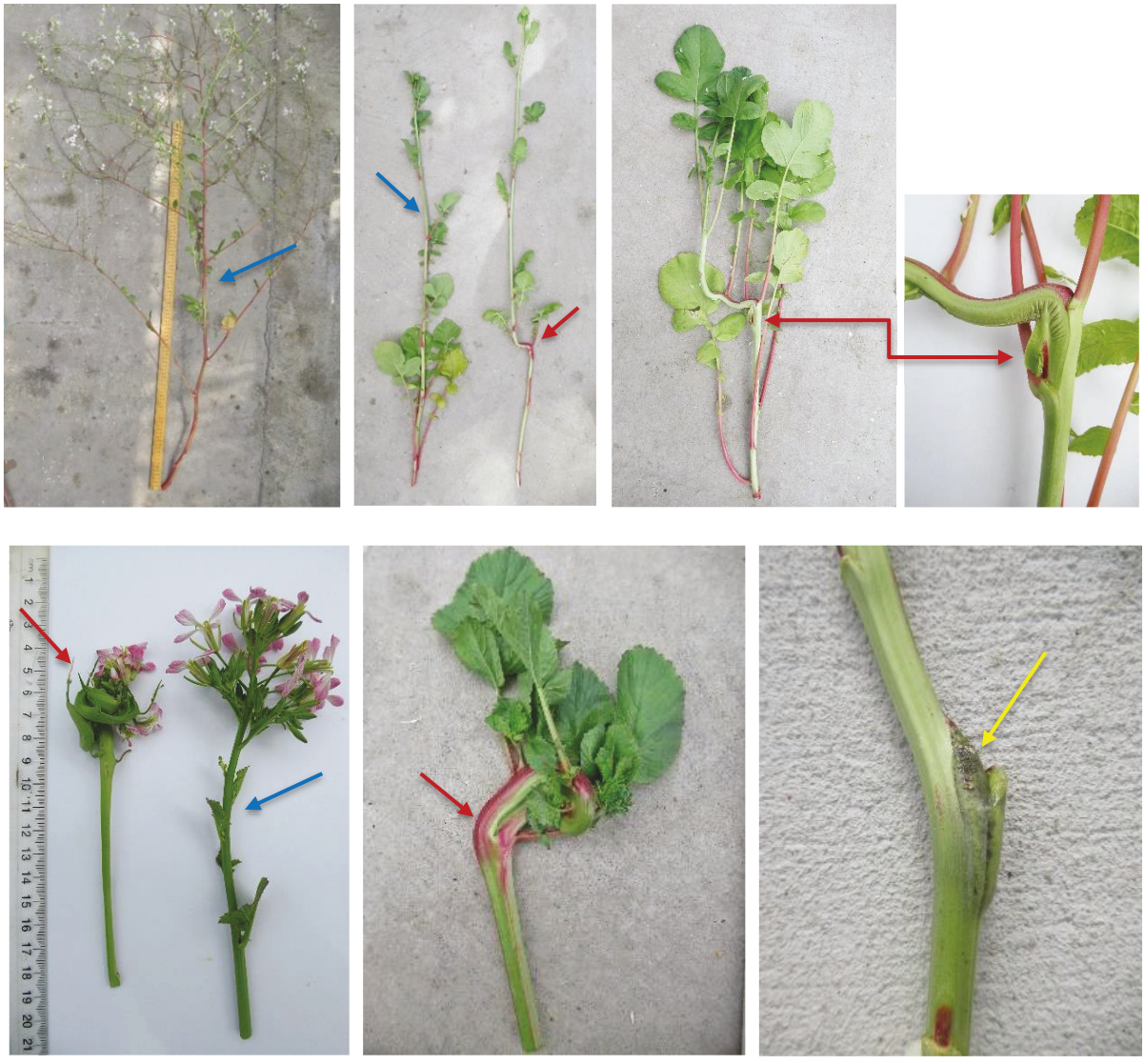


Figure 4.6. Samples of whole radish plants (blue arrows) and segments with abnormal plant parts (red arrows), gall on a stem (yellow arrow). Photos by author.

The plant was cut at the bottom of the stem with pruning shears (Fig. 4.7A). Two to five segments per plant were assessed: 10cm segment of the bottom of the main stem, 10cm segment of the top of the main stem, 10cm segment of the inflorescence (Fig. 4.7B, 4.7C) and a segment including an abnormal plant part (Fig. 4.6). To assess the endophytic growth of *A. candida*, samples were divided into two groups (i) washed with 5% sodium hypochlorite to remove any likely contaminants on the tissue surface and seeds, and (ii) not washed. All samples were then stored in a freezer until used for DNA extraction. The DNA extraction protocols and PCR amplification and sequencing were conducted as previously described.

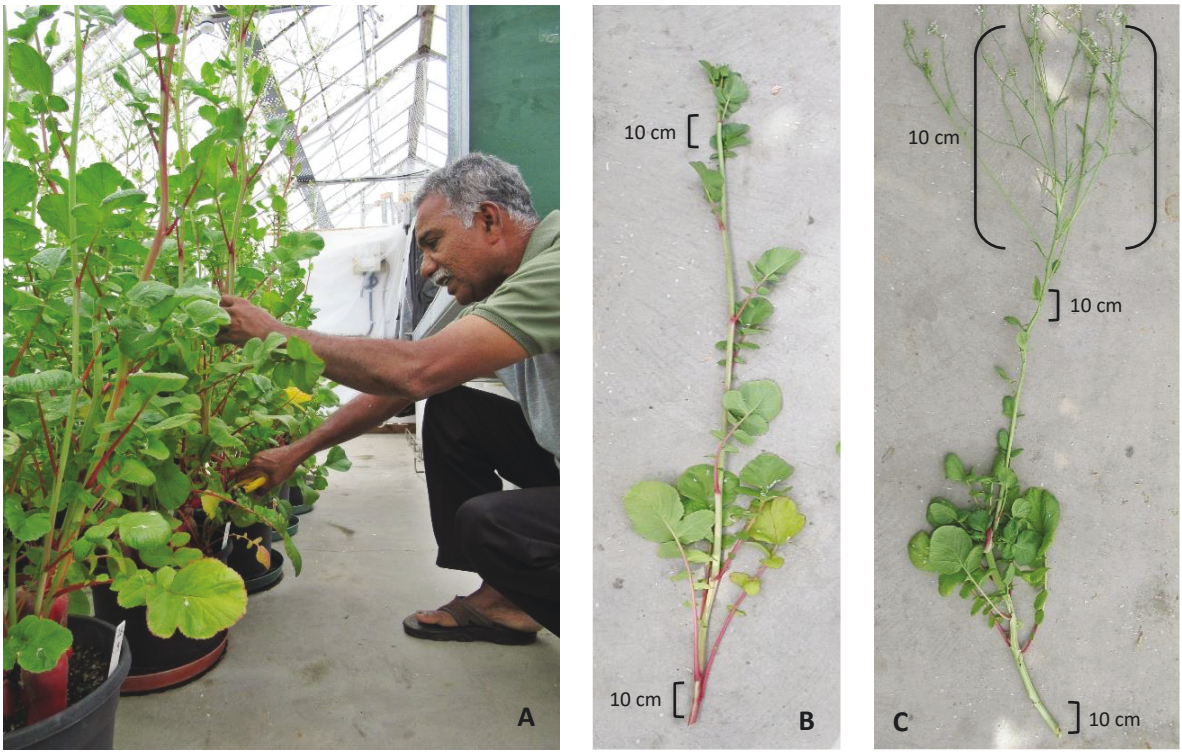


Figure 4.7. Method (A) and sampling scheme (B, C) of a radish seed plant at the glasshouse. Photos by author.

4.3 Statistical analysis

All data were analysed using an analysis of variance (ANOVA) for RCBD, using Genstat 19th edition (VSN International, Hemel Hempstead, UK). The unrestricted least significant difference (LSD) procedure at 5% significance level was used to test the differences among treatments.

4.4 Results

4.4.1 Presence and quantification of oospores on seeds

Albugo candida was found on all the seed lots tested, with infection levels ranging from 3 to 34 oospores per gram of seed (Table 4.3). The greater infection of *A. candida* was from the treatments 1, 8 and 12 with a mean of 34, 32 and 28 oospores per gram, respectively ($P<0.05$) (Table 4.3). The mean number of oospores per seed of all treatments was very low with a mean of fewer than 0.5 oospores per seed.

Table 4.3. The number of oospores on radish seed samples.

Treatment	Mean number of oospores/ 5gram of seed	Mean numbers of oospores per gram of seed	Mean number of oospores per seed
1	167.5 ^{c}	33.5 ^{c}	0.40 ^{c}
2	67.5 ^{ab}	13.5 ^{ab}	0.17 ^{ab}
3	34.5 ^{a}	6.9 ^{a}	0.08 ^{a}
4	27.5 ^{a}	5.5 ^{a}	0.07 ^{a}
5	45.0 ^{ab}	9.0 ^{ab}	0.11 ^{ab}
6	16.5 ^{a}	3.3 ^{a}	0.04 ^{a}
7	52.5 ^{ab}	10.5 ^{ab}	0.13 ^{ab}
8	161.0 ^{c}	32.2 ^{c}	0.37 ^{c}
9	50.0 ^{ab}	10.0 ^{ab}	0.12 ^{ab}
10	54.0 ^{ab}	10.8 ^{ab}	0.13 ^{ab}
11	107.5 ^{bc}	21.5 ^{bc}	0.26 ^{ab}
12	139.5 ^{c}	27.9 ^{c}	0.34 ^{c}
LSD (5%)	63.2	12.6	0.03

Within a column, treatments with a letter in common do not differ significantly ($P<0.05$). Seeds were harvested from field plots that had received their foliar fungicides applications (see Chapter 3).

Treatment: (1) Control; (2) 2.5 kg/ha Ridomil Gold MZ WG; (3) 4 kg/ha Ridomil Gold MZ WG; (4) 2 L/ha Cobra; (5) 0.4 L/ha Metalaxyl-M; (6) 3.2 l/ha Max CL; (7) 5 l/ha Foschek + 0.4 l/ha Metylaxyl; (8) 350 ml/ha Zorvec; (9) Ranman + Mancozeb + Amistar f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb f.b Ridomil Gold + Pristine f.b Ridomil Gold; (10) Ridomil Gold + Amistar f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb; (11) Ranman + Mancozeb f.b Ranman + Mancozeb + Seguris Flexi f.b Ranman + Mancozeb + Amistar f.b Ridomil Gold + Pristine f.b Ridomil Gold; (12) Ridomil Gold f.b Ridomil Gold + Seguris Flexi f.b Ridomil Gold + Amistar f.b Ranman + Mancozeb + Pristine f.b Ranman + Mancozeb. (f.b = followed by).

4.4.2 Molecular identification of *Albugo candida*

Albugo candida was found in all infected samples of radish, turnip and shepherd's purse (Table 4.4). The DNA yields from most of the samples using both methods showed bands after gel electrophoresis of the amplification products (Fig. 4.8 and 4.9).

Sequence data generated from the PCR run with primers ITS 1 and ITS 4 matched with the host plant *R. sativa* rather than the pathogen, suggesting that those pair of primers could amplify plant DNA. However, when all DNA extracted using Chelex® from sori on infected radish, turnip and shepherd's purse tissue was amplified using primers PKG-F (5'-GCCGTCGACGTGATATCTTTGC-3') and PKG-R (5'-GCGATCACATCGGCTTGTCGTGG-3') the data matched to *A. candida* suggesting that this region is not present in the plant (Table 4.4). DNA template extracted using Chelex® from the infected bulb, root and seed failed to be amplified and no band was obtained on a gel suggesting that the method is not suitable for use for these particular parts (Table 4.4).

Table 4.4. Presence of *A. candida* in sori collected from infected plant parts and lesioned plant tissues by different DNA extraction methods.

Place of collection	Host plant species	Sample	DNA extraction method	
			Chelex®	Kit
Field trial	Radish seed crop	Leaf	X	X
		Pod	X	X
		Bulb	0	X
		Staghead	X	X
		Root	0	X
		Seed	0	X
		Stem	X	X
Glasshouse trials	Radish seed crop	Leaf	X	X
		Pod	X	X
		Stem	X	X
Seed production field	Turnip seed crop	Stem	X	X
Field	Shepherd's purse	Stem	X	X

(0 = *A. candida not present;* X = *A. candida present*).

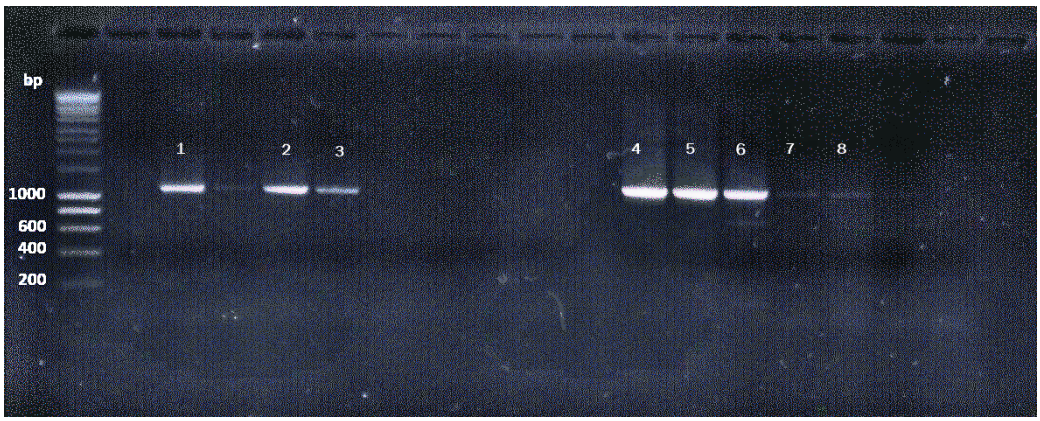


Figure 4.8. PCR amplification of PKG genes of *A. candida*. Samples from radish leaf's sori (lane 1), shepherd's purse lesioned tissue (lane 2), shepherd's purse (lane 3), radish lesioned pod (lane 4), shepherd's purse lesioned tissue (lane 5), radish lesioned bulb.

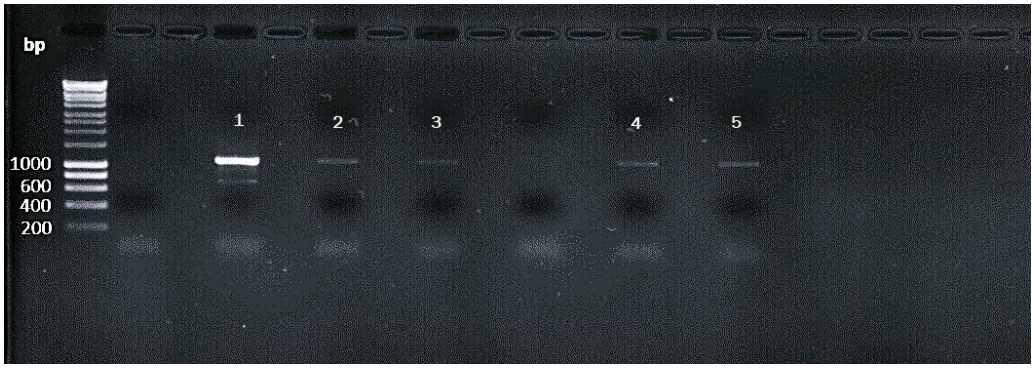


Figure 4.9. PCR amplification of PKG genes of *A. candida*. Samples from radish leaf's sori (lane 1 &2), turnip lesioned tissue (lane 3), radish pod's sori (lane 4) and radish stem's sori (lane 5). The amplified fragments represent 1080 bp when analysed by electroph.

Comparison of the gene sequences obtained from samples with the sequences available in databases revealed that all of them are related to *A. candida* with similarity from between 96 to 99% (Table 4.5). Especially, the fungi collected from radish and shepherd's purse were identified with high similarity of 99% and 98% to *A*. *candida*, respectively.

The pathogen was not found in washing seeds and stems with 5% sodium hypochlorite - suggesting that *A. candida* is not carried internally (Table 4.6). However, *A. candida* was detected on the shrivelled seeds when seeds were soaked in Tween 20 and on the galls on the stems without washing and washing with 5% sodium hypochlorite (Table 4.6).

Table 4.5. The similarity of the samples sequence data collected from different host plant species to *A. candida* from databases.

Sample	Source	Similarity (%)
Radish	Fungicide trial	99
	Glasshouse trials	99
Shepherd's purse	Field	98
Turnip	Production field	96

Table 4.6. Presence of *A. candida* in seeds and various abnormal plant parts detected by different washing methods.

Location of sample collection	Host plant species	Sample	Washing method		
			Tween 20	5% sodium hypochlorite	Not washed
Field trial	Radish seed crop	Shrivelled seeds	X	0	
		Non-symptomatic seeds	0	0	
Glasshouse trials	Radish seed crop	Gall on stem		0	X
		Abnormal plant parts		0	0

(0 = *A. candida not present;* X = *A. candida present*).

4.5 Discussion

4.5.1 Vertical transmission of *A. candida* in radish seed

While *A. candida* has often been recorded on *Brassica* seeds (Petrie, 1975, 1986; Jacobson *et al*., 1998; Armstrong, 2007; Minchinton, 2007; FAR, 2017) whether it is carried inside the seed has not been confirmed. In this experiment, *A. candida* was found on the surface of seeds collected from the field trial (see Chapter 3). These results are similar to those reported by Armstrong (2007) on *L. oleraceum*, Jacobson *et al*. (1998) on three wild Brassica species namely *L. campestre*, *Arabis lyrata* and *Erysimum menziesii eurekense,* Petrie (1975, 1986) on *R. sativus* and Petrie (1978) on turnip rape.

The method used in this study by using the PCR primer pairs PKG successfully found *A. candida* from shrivelled radish seeds after washing with Tween 20. However, after radish seeds were surface sterilised by soaking with 5% sodium hypochlorite, *A. candida* was not detected. This strongly suggested that *A. candida* is not an internally seedborne pathogen and therefore is

unlikely to be transmitted vertically through seed. However, Armstrong (2007) reported vertical transmission of *A. candida* in cress (*L. oleraceum*) seeds, but did not mention how or if the seeds were surface - sterilised. Thus, if Armstrong (2007) used the Tween washing method described in Petrie (1975), then detected *A. candida* in the seed water wash as other authors did previously (Petrie, 1975; Jacobson *et al*., 1998; Minchinton *et al.,* 2004; Minchinton, 2007; FAR, 2017) or detected *A. candida* from the seed after Tween washing, he may not have removed all the oospores from the seed surface. According to Table 4.6, *A. candida* was not detected in shrivelled seeds after 5% sodium hypochlorite washing but was detected by the Tween washing. Therefore, it appears that Tween could not remove all the oospores from on the radish seed coat whereas the 5% sodium hypochlorite did so. From these results, *A. candida* is not vertically transmitted in radish seed.

4.5.2 Presence of *A. candida* in radish and other *Brassica* crops

In New Zealand, white blister was first reported on *B. oleracea* (cabbage) in 1906 and *B. oleracea* (cauliflower and Brussels sprouts) in 1922 (Pennycook, 1989), *L. sativum* in 1880, *B. rapa* in 1919, *C. bursa-pastoris* in 1924, *M. maritima* in 1930, *Cardamine* sp. in 1943, *L. oleraceum* in 1945, *C. spinosa* in 1952, *B. campestris, L. ruderale, R. islandica* and *S. officinale* in 1953 (Baker, 1955) and *R. sativus* in 1946 (Baker, 1955; Boesewinkel, 1977). Moreover, *A. candida* is also a serious threat to New Zealand's coastal cress (*L. oleraceum*) (Armstrong, 2007). In this chapter, once again the presence of *A. candida* in the radish seed crop was confirmed.

To confirm the presence of *A. candida*, a molecular method using PCR primers PKG-F and PKG-R was used. The advantage of these primers is that they do not amplify plant genes which could be used to amplify *A. candida* DNA present in plants and also distinguish between *A. candida* and other *Albugo* species (Jouet, 2016). However, the descriptive primers in this study differ from most of the exploratory studies, which used either PCR primer pairs ITS1 + ITS2 and ITS1 + Ac13 (Jacobson *et al*., 1998; Armstrong, 2007), AC13 + AC28 (Minchinton *et al*., 2004) or ITS1 + COX2 (Choi *et al*., 2007). ITS1 + ITS4 (Riit *et al*., 2016; Alizadeh *et al*., 2017) used for identification of other fungi, could not be used in this study as they amplified plant DNA. The sequence data generated from amplification and amplicon sequencing of a pathogen isolated from turnip showed 96% similarity with *A*. *candida* sequence in the database. This is reasonably low and the pathogen might be a new species. Future work using other primers needed (Riit *et al*., 2016).

For DNA extraction, the Chelex® method showed good potential to be used for extraction of DNA from lesioned plant tissue and sori of the infected plants. The benefits of this method are the short time required, simple equipment, low cost and a sample of only 3-5 gram. The Chelex® method was effective on most of the samples assessed but did not work on infected bulbs, roots or seeds. This might be because of the presence of some PCR inhibitors in the reaction which Chelex® was not able to remove. Jacobson *et al*. (1998) used the Chelex method to detect *A. candida* in lesioned leaf tissue of *L. campestre*.

The Genomic DNA Mini Kit method detected *A. candida* in lesioned plant tissues, bulbs, roots and on shrivelled seeds successfully. The disadvantage of the Kit method is its high cost and preparation time. It takes approximately 20-30 minutes for one sample but the genomic DNA is very pure. The Kit method is easy to use and simply requires following the manufacturer's instructions. The Kit method has been previously used for detecting *A. candida* (Minchinton *et al*., 2004) and other pathogens in plant tissues (Abdel-Latif & Osman, 2017).

While several papers related to *A. candida* on Brassica seeds have been published (FAR, 2017; Armstrong, 2007; Jacobson *et al*., 1998; Petrie, 1975, 1986), there is a lack of study on the presence of *A. candida* in bulbs and roots. In this study, PCR was used to confirm the presence of *A. candida* on infected plant bulbs and roots. The origin of the *A. candida* in these samples was not clear as to whether they were from soil or the seed. However, Uma & Bhowmik (1988) and Verma & Petrie (1975) did not find any oospores in infected soil. In this study, the soil was not checked for the presence of oospores, but Agrios (2015) reported that *A. candida* could persist in the soil for a long time. Therefore, the *A. candida* found could either have come from the soil or seed. Further research should be carried out to confirm the presence of *A. candida* in non-symptomatic bulbs and roots, as the bulbs and roots used in this investigation were completely degraded and colonised.

4.5.3 Transmission of *A. candida* in the radish stem

Albugo candida was not detected in the stems, galls or abnormal plant parts (Fig. 4.6, red arrows, and yellow arrow) washed with 5% sodium hypochlorite. However, *A. candida* was identified in the galls (Fig. 4.6, yellow arrow) on unwashed stems, a result similar to the report by Minchinton *et al*. (2004) on broccoli seedlings, suggesting that *A. candida* only exists on the plants externally. Thus, *A. candida* was not transmitted systemically within the stems to cause the stagheads found in the radish seed crop. Infection of the seed head appears to be external

only, with oospore spread assisted by irrigation and wet weather (Lakra & Saharan, 1989; Minchinton, 2005; Harvey, 2006; Arora *et al*., 2019). This result was also supported by the glasshouse experiments carried out on radish (see Chapter 2), where disease incidence was greater in plants that had been overhead irrigated (foliage wet) in comparison with the plants watered at ground level (foliage dry).

4.6 Conclusions

Albugo candida was detected only on the surface of the seeds, not inside. It appears to be externally seedborne only.

The molecular methods used in this study were effective confirming the presence of *A. candida* on the infected leaves, stems, stagheads, pods, roots, bulbs and seeds of radish, and on the stem of infected turnip and shepherd's purse plants.

Chapter 5

General discussion

White blister disease is caused by *A. candida* – an obligate biotrophic parasite. *Albugo candida* affects a wide range of *Brassica* crops in the world (Saharan & Verma, 1992; Minchinton, 2007; McMullan *et al.,* 2015; CABI, 2017). In New Zealand, the presence of *A. candida* was first recorded in 1880 (Baker, 1955). Radish is one of the most important vegetable seed exports in New Zealand, with a value of $25.1 million in 2018 (NZ Horticulture, 2018). *Albugo candida* was first recorded in radish in 1945 (Baker, 1955). Now, despite extensive efforts to control *A. candida*, 20-70% seed yield losses have been reported, depending on the season (Taylor, pers. comm., 2019).

The aims of this study were to investigate the effectiveness of various methods for control of *A. candida* in radish. According to previous studies, metalaxyl had been used as a seed treatment, and mono-fungicide and multi-fungicide foliar applications showed good results for control of *A. candida* as well as increased seed yield (Braithwaite *et al*., 2018). Understanding of the transmission route of *A. candida* in radish is not well-known. Therefore, this study was conducted to (i) evaluate different seed treatment methods; (ii) to assess the effectiveness of different fungicide applications on white blister disease, seed yield and quality, and (iii) to investigate whether *A. candida* can survive within the plant and be transmitted vertically through radish seeds.

Seed treatments of infected radish seeds assessed were hot water (50^{o}C for 15, 20, or 25 mins), *T. atroviride* (strains LU132 and LU140), and fungicides (Ridomil Gold MZ WG, Thiram and Iprodione). None of the seed treatments in this study provided control of *A. candida* in radish seeds. It is possible that the temperature of hot water at 50^{o}C was not high enough to kill oospores on the radish seed coat, although 50^{o}C has been used for control of *Alternaria* spp. and *Phoma lingam* in seeds of Brassica species (Holtzhausen, 1978; Nega *et al.,* 2003; Toporek & Hudelson, 2017). Therefore, further research about a suitable temperature and time are required. Likewise, *T. atroviride* strains LU132 and LU140 did not control *A. candida*, but *T. atroviride* strain LU132 does control *R. solani* and *G. graminis* var. *tritici* (Lee, 2018; Umar, 2018). Fungicides Thiram and Iprodione were reported to be effective seed treatments in mustard and radish for control of *A. candida*, *Erysiphe crucilerarum* and *Alternaria* spp.

(Holtzhausen, 1978; Dange *et al.,* 2003; Islam *et al*., 2007). Verma & Petrie (1979), Stone *et al*. (1977) and Saharan *et al.* (2014) had reported that metalaxyl plus mancozeb reduced *A. candida* significantly. In particular, the combination of metalaxyl seed treatment followed by metalaxyl plus mancozeb foliar fungicide controlled *A. candida* effectively (Stone *et al*., 1987; Bhargava *et al*., 1997; Minchinton *et al.,* 2004). Moreover, the seedling emergence in both experiments was not high, especially for experiment 2. Why all the seed treatments used failed to prevent seedling infection is not known and requires further investigation.

In the field trial, once again, none of the fungicides increased seed yield or seed quality. In the 2018-2019 season, the single fungicides Ridomil Gold MZ WG, Cobra and Max CL, and multiple fungicides Foschek plus Metylaxyl-M showed their potential for control of *A. candida.* Similarly*,* fungicides Max CL and Foschek reduced white blister disease in the 2013-2014 and 2016-2017 seasons, respectively (FAR, 2016; 2017). Also, Patnude & Nelson (2013) and Braithwaite *et al*. (2018) reported that Ranman controlled white blister effectively. Metalaxyl-M plus mancozeb has been used as the standard fungicide for control of *A. candida* in New Zealand (FAR, 2016; 2017; Braithwaite *et al*., 2018) and in the world (Verma & Petrie, 1979; Mathur & Bhatnagar, 1991; Minchinton *et al*., 2004; Patnude & Nelson, 2013; CABI, 2016) but there is concern about the development of resistance following continued use. The rotation of multiple fungicides in treatments 9 (Ranman plus Mancozeb plus Amistar followed by Ranman plus Mancozeb plus Seguris Flexi, then Ranman plus Mancozeb, then Ridomil Gold plus Pristine and finally Ridomil Gold) and 11 (Ranman plus Mancozeb followed by Ranman plus Mancozeb plus Seguris Flexi then Ranman plus Mancozeb plus Amistar, then Ridomil Gold MZ WG plus Pristine and finally Ridomil Gold MZ WG) showed limited control of *A. candida*. The fungcide Amistar or combination fungicides Amistar plus Ridomil or Amistar plus Ridomil Gold MZ WG has previously been reported to significantly reduce white blister disease (Minchinton *et al.*, 2004; Patnude & Nelson, 2013; FAR, 2017; Gairola & Tewari, 2019). Why white blister control and radish seed yield were not improved by any of the fungicide treatments used in 2018-2019 season is surprising, and cannot be explained.

Other oomycetes pathogens have started to become resistant to Metalaxyl (Cook & Zhang, 1985; Molinero-Ruiz *et al*., 2003; Matson *et al*., 2015) and *A. candida* race 1 can resist Metalaxyl (Valdes & Edgington, 1983; Liu, 1992). The race of *A. candida* causing white blister disease on radish in New Zealand is not yet known. If it is race 1, then to reduce the risk of

possible fungicide resistance from *A. candida*, further research about the use of non-chemical control measures is required.

Radish seeds collected from the field trial were found to carry *A. candida* on the seeds. After washing with 5% sodium hypochlorite, no *A. candida* was detected in radish seeds, abnormal plant parts, stems or galls on the stem. However, *A. candida* was detected on shrivelled radish seeds after washing with Tween 20, and from galls on the stems without washing. This indicated that Tween could not remove all oospores from the surface of seeds. Therefore, based on these results, *A. candida* is only externally seedborne in radish and is not transmitted internally. In this study, the bulbs and roots were not surface sterilised. Thus, the *A. candida* detected from bulbs and roots could have come from soil, infected seeds, plant debris in the field or inside bulbs and roots.

It is likely that seedborne inoculum is the major source for establishment of white blister disease in radish seed crops. None of the seed treatments used in the two glasshouse experiments prevented disease transmission, but in the PCR study, washing seeds with 5% sodium hypochlorite killed the externally borne oospores. This suggests two options for the industry. The most preferable would be the provision of seeds of radish parent lines that do not carry *A. candida*. If this is not possible, then an effective method to kill seedborne oospores is required. Surface sterilisation by washing with 5% sodium hypochlorite was successful with a small quantity of seeds in the laboratory. Whether this can be done safely for a commercial seed lot requires investigation. Hot water treatment is another method that requires further evaluation. It may be, for example, that 50^{o}C was insufficient to kill the oospores. Finding the balance between a temperature able to do so, but without harming germination, will need to be assessed. Agrios (2015) reported that oospores survive in the soil and plant debris for many years whereas Verma & Petrie (1975) and Verma *et al*. (1988) did not find oospores in the soil. These conflicting reports require investigation. If oospores are found in the soil, further soil treatment methods should be considered.

In this study, PCR primer pairs PKG-F and PKG-R were able to detect *A. candida* from infected tissues. Sori collected from all infected radish, turnip and shepherd's purse plants were caused by *A. candida. Albugo candida* race 1 is reported to be the pathogen of *R. sativus* (Pound & Williams, 1963; Minchinton *et al*., 2004), races 2V, 7, 7V, 35 and 36 of *B. rapa* (Verma & Petrie, 1975; Petrie, 1994; Jat, 1999; Verma *et al*., 1999; Rimmer *et al*., 2000; Minchinton *et al*., 2004;

McMullan *et al*., 2015) and races 4 and AcEm2 infect *C. bursa-pastoris* (Pound & Williams, 1963; Borhan *et al*., 2008; Jouet, 2016). In New Zealand, *A. candida* is reported race 7 in *B. rapa* and race 9 in *B. oleracea* have been reported (Kaur, 2013). However, different *A. candida* races have been found on the same species depending on the collection places. For example, *B. juncea* from North America is race 2 (Pound & Williams, 1963) while *B. juncea* from India is race 12 (Verma *et al*., 1999). Therefore, identification of the races of *A. candida* found in New Zealand is required. Also, whether the same races can occur on different *Brassica* crops in New Zealand requires investigation.

The current glasshouse study showed that irrigation method allowed different levels of disease infection in radish. Overhead irrigation increased the disease incidence and severity more than the watering into trays. One of the ideal conditions for sporangia germination is free water. So under overhead watering, more zoospores are released to infect other plants (Liu, 1992). A field trial to confirm this using different irrigation methods (drip and overhead) is highly recommended.

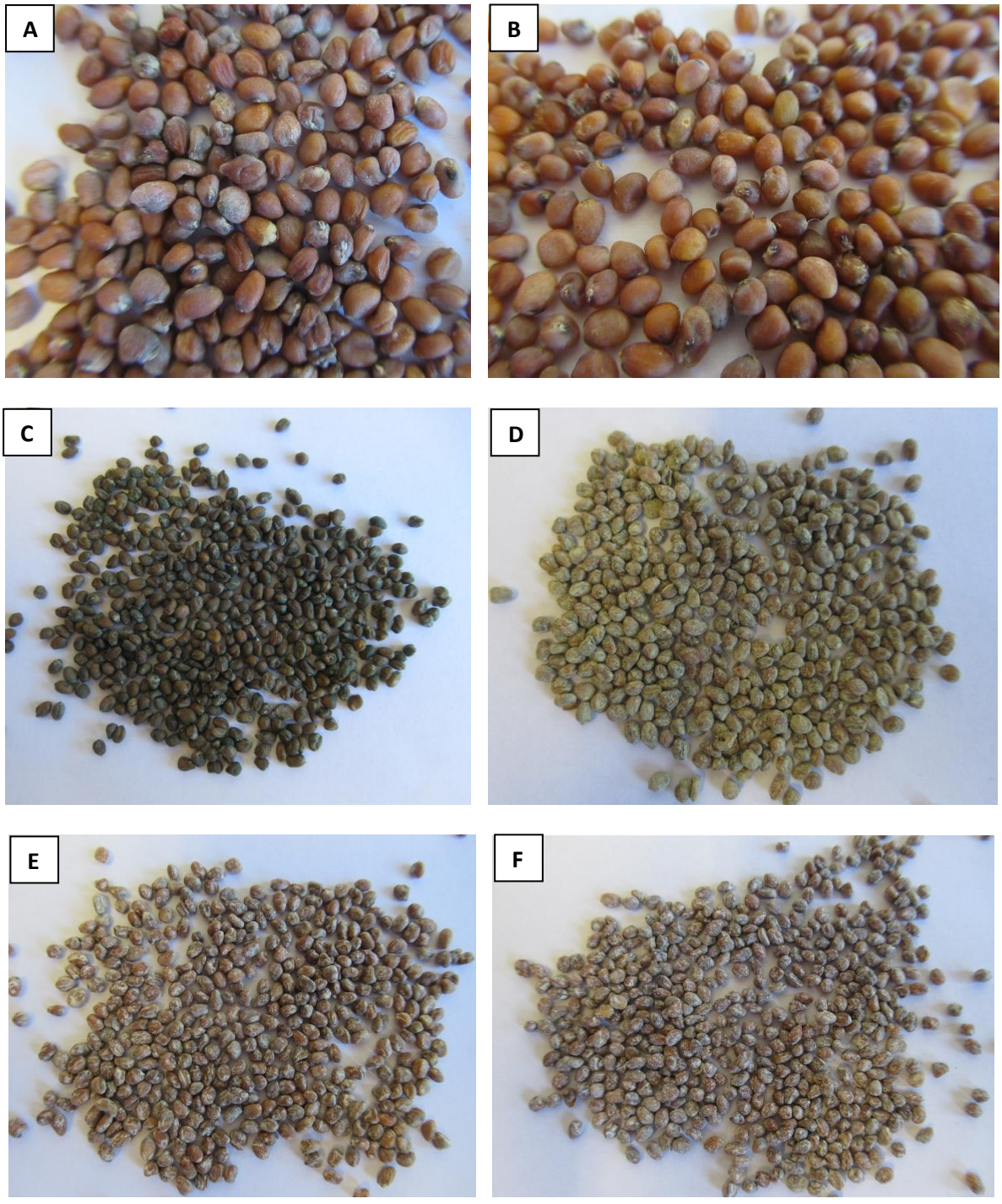
The disease assessment in this study was based on Braithwaite's disease score of the white blister on radish which described symptoms on the leaf without images (Braithwaite *et al*., 2018). Thus, a disease assessment key is required for seedlings, pods, stagheads, stems, bulbs and roots.

In the 2018-2019 season, the average cost of fungicides for each of the treatments was $490/ha, but seed yield was not increased. While the seed yields were higher than in previous years (e.g. in the 2016-2017 season, the seed yield for the Ridomil Gold MZ WG treatment ranged from 130 g/m^2 to 135 g/m^2 (FAR, 2017; Braithwaite *et al*., 2018) whereas in the present trial, it was 157 g/m^2, the cost of the fungicide application did not provide a return on the investment.

Appendix A

Radish seeds

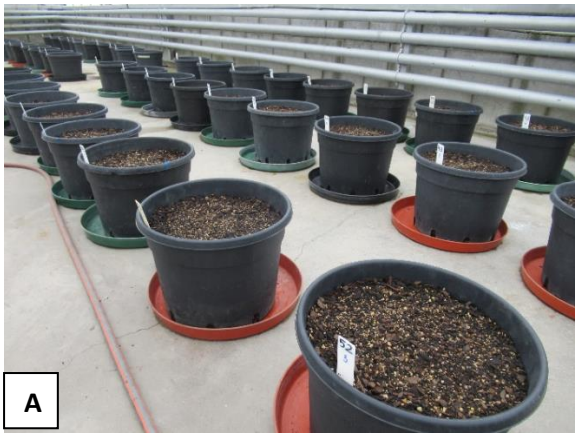
(A) Untreated, (B) Treated with hot water 50°C, (C) Treated with *Trichoderma atroviride*, (D) treated with Metalaxyl and mancozeb, (E) Treated with Thiram and (F) Treated with Iprodione. Photos by author.



Appendix B

Experiment 1 in the glasshouse

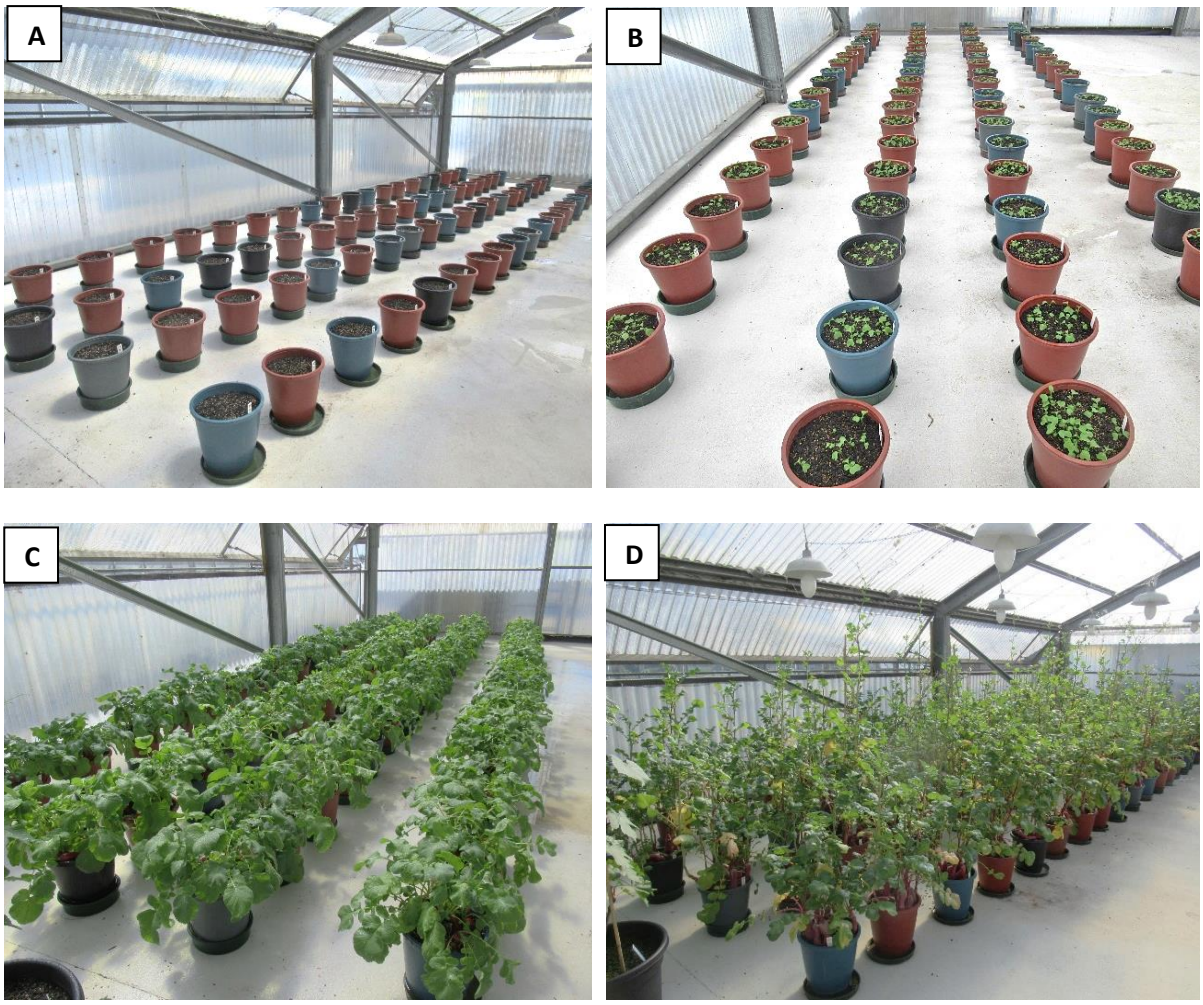
(A) Sowing date, (B) 10 DAS, (C) 75 DAS, (D) 86 DAS, (E) 116 DAS. Photos by author.



Appendix C

Experiment 2 in the glasshouse

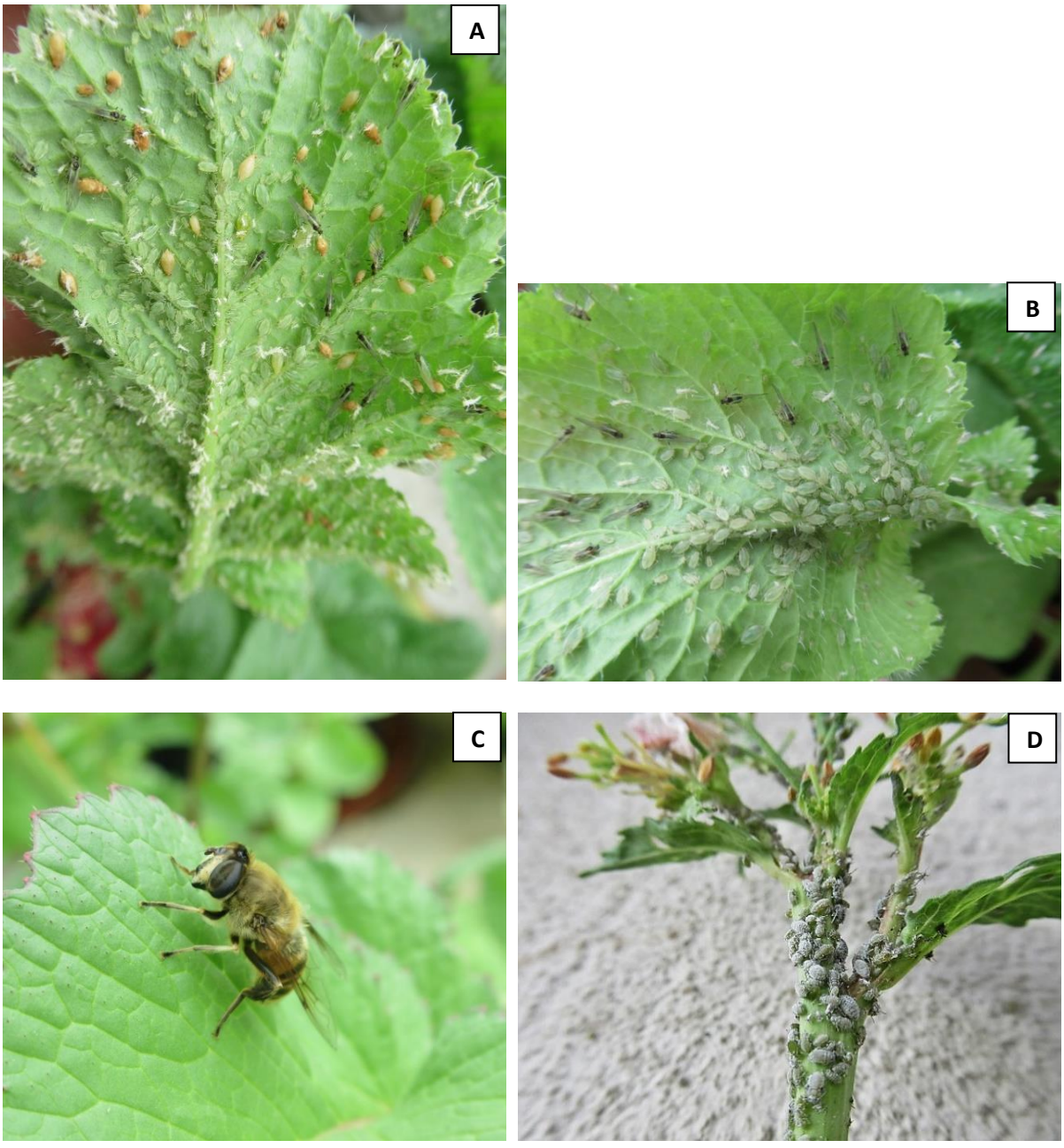
(A) Sowing date, (B) 16 DAS, (C) 48 DAS, (D) 129 DAS. Photos by author.



Appendix D

Insects on a radish plant in the glasshouses

(A, B) aphids, (C) bee, and (D) mealy bugs. Photos by author.



Appendix E

Establishing whether *Albugo candida* is transmitted from seed to seedlings

E.1 Introduction

Albugo candida causes damage to radish seed crops and reduces seed yield in New Zealand (FAR, 2017). The pathogen can be seed transmitted. Jacobson et al. (1998) reported that *A. candida* was detected in a high proportion of *Lepidium campestre, Arabis lyrata*, and *Erysimum menziesii eurekense* seedlings when seeds harvested from infected plants were sown. However, Minchinton (2007) found no white blister symptoms were observed in mustard seedlings that were planted from oospore infested seeds. Therefore, the aims of the present study were to i) assess emergence of seedlings from seeds with various levels of oospore contamination in the glasshouse and ii) demonstrate seed transmission by assessing disease incidence at 80 DAS.

E.2 Materials and methods

The experiment was carried out at the glasshouse, Lincoln University from 9th May 2019 to 7th August 2019. Radish seeds from the field trial (Chapter 3) were checked for oospore contamination using the method described in Chapter 4. Seed lots were then divided into three groups: high mean numbers of oospores per gram of seed (>20), medium (10-20) and low (<10). Seeds chosen randomly from two size groups <2.6mm and 2.6-3mm from the control treatment of the field trial were also used in this experiment. Healthy seeds with no oospore detected (size 2.6-3mm) were used as a control in this experiment (Table E.1).

Six replicates of each treatment were sown with ten seeds in an individual pot (10 litres) constituting a replicate (Fig. E.1). Lincoln University's standard 3-4 months potting mix was used as described in 2.2. This experiment could not be set up in a typical complete block design because of the possibility of cross contamination by oospores from seedlings of one seed lot to seedlings of another seed lot. The six replicates were placed together and physically separated from the other treatments. This was achieved by placing each treatment in a different position within the glasshouse. Water was added to the saucers in which the pots

were placed to prevent oospore spread via water splash (Fig. E.2). Temperature was recorded around 10 AM at the glasshouse every week after radish seeds were sown.

Table E.1. List of treatments used in the experiment.

No.	Treatment	Source
1	Control (2.6-3mm)	FAR
2	Seed category <2.6 mm	Field trial
3	Seed category 2.6 - 3 mm	Field trial
4	High (>20 oospores/g)	Field trial
5	Medium (10-20 oospores/g)	Field trial
6	Low (<10 oospores/g)	Field trial

Figure E.1. Seed transmission experiment in the glasshouse. Photo by author.

Figure E.2. Watering into the saucer. Photo by author.

Data were recorded once a week from 10 DAS until 91 DAS. Data collected were:

- Seedling emergence (%): the number of normal seedlings in each pot were counted. The result was reported as percentage of normal seedling emergence in each treatment.

- Disease incidence (%): disease incidence of each treatment was assessed by the number of plants with symptoms on leaves or stems caused by *A. candida*, following the formula in 2.3.2.

E.3 Results

Seedling emergence

Figure E.3. Seedling emergence assessment at 10 DAS. Photo by author.

Table E.2. Percentage seedling emergence at 10 DAS.

No.	Treatment	Seedling emergence (%)
1	Control	100
2	Seed category <2.6 mm	97
3	Seed category 2.6 - 3 mm	94
4	High (>20 oospores/g)	92
5	Medium (10-20 oospores/g)	95
6	Low (<10 oospores/g)	98

Emergence was evaluated at 10 DAS (Fig. E.3). Temperature during emergence ranged from 14 to 21°C. All radish seed lots had high seedling emergence ranging from 92 to 100% (Table E.2).

Temperatures in the glasshouse

Temperatures fluctuated during the experiment (Fig. E.4), reaching the highest point of 20°C in the 12th week (78 DAS), and the lowest point of 17°C in the 4th week (22 DAS). An average temperature was recorded of 18°C.

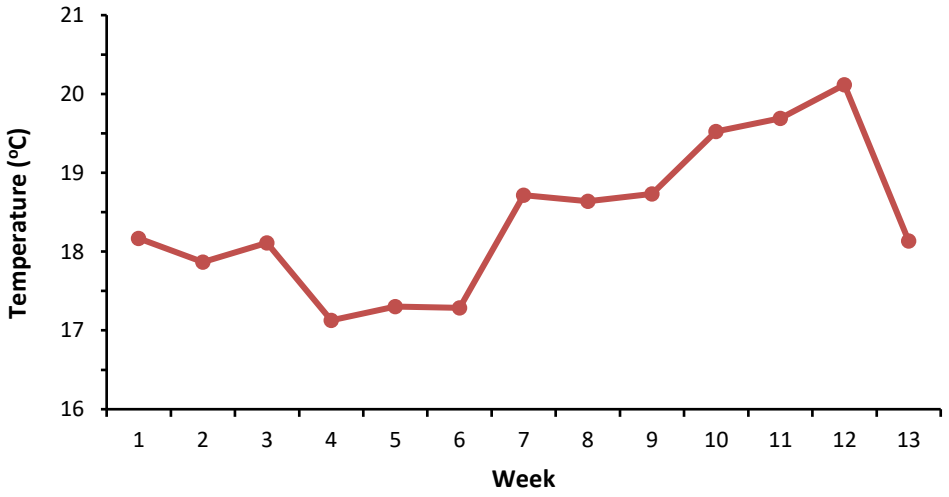


Figure E.4. The average temperature in the glasshouse through the experiment.

Disease assessment

In this study, no symptoms of white blister disease were found on any radish plants at any assessment time.

E.4 Discussion

No *A. candida* symptoms were found on any of the plants in this experiment. Therefore, the confirmation of transmission from seed to seedlings was not achieved. It could be either the environment was too cold to allow disease symptom expression or the number of oospores were insufficient for disease development. The experiment ran from 9th May 2019 to 7th August 2019. The average temperature was 18°C but night time temperatures were often <5°C. *Albugo candida* requires a mean temperature of 10-20°C for oospore germination

(Verma & Petrie, 1975; Minchinton *et al.,* 2004; Mehta, 2014). According to the results in 2.4.2.1, *A. candida* symptoms first occurred at 70 DAS in experiment 1 when temperature was higher than 22^{o}C. Thus, for temperature is the most likely a reason no disease developed.

Appendix F

Agrichemical fertilizer and irrigation applications throughout the trial period

Material	Product	Chemical group	Active ingredient	Rate	Date of application
Herbicide					
1	Treflan Nf	K1	Trifluralin	2.5 L/ha	10/09/2018
2	Alanex	K3	Alachlor	5 L/ha	23/09/2018
3	Starane Xtra	O3	Fluroxypryr	350 mL/ha	27/10/2018
				500 mL/ha	13/11/2018
4	Starane	O3	Fluroxypryr	450 mL/ha	05/11/2018
5	Versatill Powerflo	O3	Clopyralid	200 mL/ha	05/11/2018
6	Versatill	O3	Clopyralid	200 mL/ha	13/11/2018
7	Ignite	A	Glufosinate-ammonium	400 mL/ha	30/11/2018
8	Reglone	D	Diquat	5 L/ha	12/03/2019
Insecticide					
1	Lorsban 50EC	1	Chlorpyrifos	500 mL/ha	27/10/2018
				600 mL/ha	16/02/2019
2	Karate Zeon	3	Lambda - Cyhalothrin	30 mL/ha	05/11/2018
				30 mL/ha	08/12/2018
3	Exirel	28	Cyantraniliprole	150 mL/ha	04/01/2019
Fertiliser					
1	YaraVita Molytrac 250		Molybdenum	500 mL/ha	10/09/2018
2	Cropmaster 15		Compound fertiliser	250 kg/ha	11/09/2018
				190 kg/ha	10/12/2018
3	Kieserite Granular		Magnesium sulphate	200 kg/ha	11/09/2018
4	Borate 46		Boron	20 kg/ha	11/09/2018
5	Bio Marinus Biological		Fish fertiliser	15 L/ha	23/09/2018
				10 L/ha	19/12/2018
6	Nitrophoska Select		Compound fertiliser	175 kg/ha	31/10/2018
7	Brassitrel Pro		Compound fertiliser	2 L/ha	8/12/2018
Irrigation					
1	Irrigation			30 mm	08/01/2019
2	Irrigation			30 mm	26/01/2019
3	Irrigation			30 mm	03/02/2019
4	Irrigation			30 mm	09/02/2019

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